INFLUENCE OF MANAGEMENT ON HERBAGE YIELD, PERSISTENCE, CARBOHYDRATE RESERVES AND NUTRITIVE VALUE OF 'AFRICAN' AND 'FLORIDA 66' ALFALFA (*MEDICAGO SATIVA* L.)

By

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DOCTOR OF PHILOSOPHY

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TO MY KINDRED

WITH INEFFABLE AFFECTION AYE

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In 1972 and 1973 studies were conducted on various quantitative and qualitative aspects of 'African' and 'Florida 66' alfalfa. Florida 66 is a new cultivar that has been selected in, and for, Florida where other unadapted alfalfas, e.g., African, previously failed to persist.

At the 3- and 6-week cutting intervals in Experiment I, Florida 66 had greater total nonstructural carbohydrate (TNC) root reserves and, consequently, greater persistence and DM production than African. A more developed tap-root system found in Florida 66 may also contribute to plant longevity through anchorage and access to nutrients further afield.

Following each defoliation of alfalfa in the field, TNC levels dropped steadily, possibly due to withdrawal by the initial regrowth. Also in winter root TNC dropped considerably, evidently due to utilization by alfalfa

plants for respiration and maintenance through a period of little or no growth. The 3-week cutting interval severely and progressively reduced TNC, regrowth potential, stand persistence and DM yields of both alfalfas. Regression equations were developed for the estimation of current root TNC using the weight of regrowth-in-the-dark, the two of which were highly and positively correlated.

The productivity of Florida 66 at different rates and frequency of fertilization, harvesting schedules and stage of growth was studied in Experiments II, III and IV. Alfalfa's basic requirements for soil pH, Ca, Mg and micronutrients were met through liming and periodic application of fritted trace elements. There was no response to P fertilization. However, DM production and stand persistence of Florida 66 were increased by K fertilization up to the highest rate (372 kg/ha/year). Response to split applications at higher rates was evident only in the second season.

At early stages of maturity, alfalfa hay was low in neutral detergent fiber (NDF), high in crude protein (CP) and *in vitro* and *in vivo* OM digestibility, but was low in CP and digestible organic matter (DOM) yield per hectare. The highest yield of DM, CP and DOM per hectare were obtained from a 6-week fixed cutting interval. Generally, second-season Florida 66 had higher CP and IVOMD, but lower DM yield per hectare than the first-season crop.

At similar cutting intervals within a season, subsequent cuts tended to have higher NDF but lower digestible OM and CP intake and digestibility. Between maturity groups, digestible OM, CP and NDF intake and digestibility diminished with increasing maturity. Chopped hay which was used in the sheep feeding trial contained lower CP, higher NDF and was less digestible (IVOMD) than alfalfa field samples mainly due to loss of tender parts during the process of hay-making and handling. Leaf-to-stem ratio in alfalfa declined with maturity. At all the three stages of maturity (3-, 4.5- and 6-week), the leaf fraction had higher CP, IVOMD and lower NDF than the stem.

A high and positively significant ($P < .001$) correlation ($r = + .96$) was established between IVOMD and *in vivo* OMD of Florida 66. Slight under-estimation of *in vivo* OMD was unavoidably due to selection by sheep of the hay offered. There was a positive and significant relationship between digestible CP intake and % CP, digestible OM intake and OMD. The value of alfalfa as a high protein feed for livestock and an imminent source of protein for human consumption was further demonstrated by high levels of CP in Florida 66 alfalfa, even at advanced stages of maturity.

CHAPTER I

INTRODUCTION

Alfalfa is the most widely grown forage legume in the United States. More than 95% of it is fed to ruminant livestock as hay, silage, and greenchop, the intake of which is usually greater than that of grasses of equal digestibility. Alfalfa is an excellent source of Ca, Mg, P, and vitamins A and D, while its protein is reportedly better than most of the plant proteins, and nearly comparable to milk and other animal proteins. It also has a better distribution of essential amino acids than soy protein. Currently, in Wisconsin and California research is in progress toward devising and improving systems and processes of extracting protein from alfalfa, a relatively cheaper source, to be used for human consumption and in animal feed supplements.

Under Florida's hot and humid climate, previously available alfalfa varieties have acted more like annuals than perennials because of the loss of stands during the long summer period. Stands are often so sparse by the end of the first harvest season that further production becomes uneconomical (167, 179). The expense and uncertainty of establishing a new crop of alfalfa each year has further

minimized its production in the state. Thus, of all the alfalfa produced in the United States, much less than 1% is in the state of Florida. Similar decline in growth of alfalfa plants, often referred to as a "summer slump" has been reported from the warmer southwestern United States (72, 176, 177).

The persistence of alfalfa has been found to be closely related to the level of carbohydrate reserves stored mainly in the roots (81, 83, 84, 187, 244). Consequently, the failure of alfalfa to persist in Florida has been attributed mostly to the loss of carbohydrate reserves caused by the longer duration of high temperature under which plants respire at a high rate over a long period of time. Respiration is the oxidation of carbohydrates to carbon dioxide, water, and energy. Energy may in turn be used for growth or wasted as heat.

If the energy is used for growth, the faster growth brought about by the increase in respiration may result in more frequent harvests. Thus, a greater percentage of the carbohydrate from photosynthesis would go into top growth and less would be available to replace root reserves. A more frequent harvesting schedule may also remove younger plants which will not have formed a full canopy of leaves needed to completely intercept the available light energy and to synthesize enough food for growth and storage. It is also possible that under Florida's climatic conditions, alfalfa is harvested sooner after seeding, thereby reducing the time available for

root growth and food storage.

Subsequently, weakened alfalfa plants become more susceptible to diseases and other injurious factors, while the thinning stands encourage rapid ingress of weeds.

'Florida 66' alfalfa was developed under Florida's hot and humid conditions through generations of extended selection pressure, and recently released to growers in the state. In a few field trials reported so far, it has consistently outyielded and outpersisted all varieties tested, including 'African', a nonhardy and unselected variety.

The main factors contributing to Florida 66 alfalfa's excellence, be it carbohydrate reserves or other agronomic characteristics, need to be better ascertained, along with suitable management practices that would maintain or enhance its productivity and feeding value.

The investigations reported herein comprised four experiments. The first experiment was designed to study the effect of season and stage of maturity on the trend and levels of carbohydrate reserves in relation to the faculty of regrowth, herbage yield and persistence of African and Florida 66 alfalfas. The purpose of the three other experiments was to provide contemporary data on Florida 66 alfalfa's response to fertilization, cutting schedules, season and stage of maturity in terms of herbage yield, persistence, nutrient recovery, chemical composition, and feeding value.

CHAPTER II

REVIEW OF LITERATURE

The Alfalfa Plant

Alfalfa (*Medicago sativa* L.) is a herbaceous plant of the family *Leguminosae* and subfamily *Papilionoideae*. Its erect stems usually reach a height of 60 to 90 cm. There may be from 5 to 25 or more stems per plant, rising from a woody crown from which new stems grow when the older ones mature or are cut. The root system has a distinct tap root which under favorable conditions may penetrate the soil 7 to 9 m or more (88).

Alfalfa's Origin and Adaptation

Alfalfa evolved in the Near East and Central Asia, a region of late springs, short dry summers, and low humidity. The soils are well drained, typically near neutral in pH, and with a high lime content in both the soil and subsoil (34).

Under natural conditions, such as those found in its center of origin, alfalfa does well under high light intensity and cool temperatures rather than cloudy, humid and hot conditions. Alfalfa tolerates drought, but conversely it does not withstand flooding. Murata et al. (150) showed that the soil moisture could be reduced to 35% of the water

holding capacity of a soil before affecting the photosynthesis and growth of alfalfa. This inherent characteristic is in addition to the deep root system which enables alfalfa to draw moisture from soil depths below the root range of many plants (96, 113).

The first recorded attempt to grow alfalfa in the United States was in 1736 in Georgia where it had little success due to the typically shallow, poorly drained acid soils and humid climate of the eastern states (33). It was not until 1850 when it was introduced into the west coast that it really succeeded on the deep loam soils whose porous subsoils permit good internal drainage. Alfalfa requires large amounts of lime and does not do well on soils which are decidedly acid (38, 255). Alfalfa also grows extremely well in dry climates on fertile soils where there is sufficient moisture, such as in soils under irrigation.

Since its major expansion in the 19th century, better adapted and improved ecotypes of alfalfa have evolved in different areas of the world through natural and farmer-imposed selection (118).

The Development of African and Florida 66 Cultivars

Florida 66 is a non-hardy alfalfa which was developed by Horner (94) for well drained soils in Florida through extended selection pressure under humid conditions. Seed used to produce foundation seed of Florida 66 came from the planting made in 1963 and harvested in 1966, which was

the sixth generation of selection from the original 100 alfalfa varieties and introductions obtained from the U.S.D.A. at Beltsville, Maryland, and planted at Gainesville, Florida, in 1950. Florida 66 was released as a named variety in 1967.

In field trials, Florida 66 has consistently outyielded and out-persisted all other varieties tested, in the second and up to the third harvest year (94, 167, 179). These workers have reported that intensive management was the main factor in its successful production.

African alfalfa was introduced from Egypt to California in 1924 (33). The original stock was labelled as U.S.D.A., F.C. 31370 Hegazi alfalfa. Seed from selected plants, that survived during several years, was increased as African and is now maintained by the California and Arizona Agricultural Experiment Stations.

African alfalfa is reported as having very little dormancy, and that it grows late in autumn and early in the spring (33). It is adapted only in the extreme southern and southwestern United States, where stands seldom last more than three years (167, 179). The varieties Moapa and Sonora were selected from African alfalfa (89).

Alfalfa Nutrition and Fertilizer Use

Introduction

Until about 1960 alfalfa yields in the United States rarely exceeded 5 tons/acre. Recently, yields of from 8

to 16 tons/acre have been reported (5, 42, 67). Increased use of fertilizer, among other practices, has been cited as being responsible for the increased yields.

When alfalfa is managed for maximum production, more frequent cutting results in higher yields, higher quality, and greater nutrient removal (218). More frequent cutting also results in harvesting of younger plants that have a higher concentration of mineral elements (29). In turn, this has increased the need for more fertilizer use in order to replace nutrients in the soil which are periodically lost through heavy crop removal (28, 218).

Soil Acidity and Liming

The optimum pH for alfalfa production varies considerably, depending on soil texture, organic matter, and Ca and Mg content. Under most situations a soil/water pH value between 6.5 and 7.5 has been reported as being ideal for maximum alfalfa production (38, 255).

The main purpose of liming is to correct soil acidity. In addition, liming provides Ca (from agricultural lime) or Ca and Mg (from dolomitic lime), as plant nutrients. Calcium promotes root development of alfalfa, and is essential for nodulation and N-fixation (242). In acid soils, Pohlman (165) reported that fibrous feeder roots and nodules were confined to the limed layers. Liming reduces the solubility of Al and Mn, which are believed to be the primary cause of poor growth of alfalfa in acid soils (242). Liming increases the availability of Mo (117) and P (242). Overliming

may decrease the availability of P (255).

Although alfalfa takes up large amounts of Ca, deficiency of this element under field conditions is said to be rare (252).

Nitrogen

The N level of healthy alfalfa plants at first flower has been reported to be around 3% (152), the younger portions of the plant containing a higher percentage of N than the older portions.

Properly inoculated alfalfa will fix large quantities of atmospheric N (45). Thus, N is seldom applied to pure alfalfa stands, although, on poor soils, a small amount at seeding time may be necessary to enhance seeding growth until rhizobia in root nodules are able to fix atmospheric N (18). The general conclusion is that, alfalfa and alfalfa-grass mixtures composed of one-third alfalfa or more do not respond markedly to N fertilization (173).

Phosphorus

The P concentration in oven-dry alfalfa has been reported to be in the range 0.2 to 0.40%, the critical level being around 0.25% (24, 78, 152). Acid soils contain large amounts of active Fe and Al, while alkaline and calcareous soils contain Ca. The more acid the soil is, the less soluble the iron and aluminum phosphates will be. Calcium phosphates begin to form at about pH 6.0, where they are most soluble,

and their solubility decreases as the pH increases. Thus, liming can have a pronounced influence on availability of soil P (163).

Rate of application of P fertilizer depends largely on the amount of available P in the soil and the yield level of the alfalfa. Phosphorus is not lost from most soils by leaching and the amount removed by alfalfa, especially immediately after application, is low and usually in the range of 10 to 30% of that applied (67).

Phosphorus is absorbed very rapidly by young plants. Seatz and Stanberry (183) stated that by the time a young plant has attained about 25% of its total dry weight, it may have already accumulated as much as 75% of its total P. Levesque and Katcheson (125) have stressed the importance of P availability for survival of alfalfa seedlings in the 4- to 6-week stages of growth when soil temperatures are low. However, there appears to be no yield advantage in later harvests (50, 100).

Phosphates are relatively immobile in most soils. Depth of penetration of P appears to be related to rate of P applied and to soil texture (90, 186). Sandal and Garey (181), using 40 lbs/acre of P_2O_5 increased the P slightly in the 2- to 3-inch layer, while an annual application of 120 lbs of P_2O_5/acre increased P in the top 15 cm. Drake and Stewart (75) obtained higher yields of alfalfa from deep placement at 20 cm, in addition to surface banding of

P at seeding time. Also, with deep placement of P, less wilting of alfalfa plants was observed during dry weather. However, top dressing results in less fixation, since applied P comes in contact with a smaller quantity of soil than P which is disked in. The effectiveness of top dressed P is apparently due to the zone of high root activity of alfalfa plants near the soil surface (75, 99) and the absorption of some of the P by the crown (220).

Studies have indicated that there does not appear to be a great deal of difference in the response obtained from applying a large initial application of P as compared to smaller annual applications (93, 215).

Alway and Nesom (4) and Nelson and MacGregor (153) reported an increase of about 1% crude protein in alfalfa with the addition of P fertilizer, especially in deficient soils. However, Hoff and Dotzenko (93) and Schmehl and Romsdal (182) reported no effect on percent crude protein, although dry matter yields were significantly increased.

Potassium

Potassium is present in alfalfa in a higher concentration than any other mineral element with the possible exception of N. Recent studies have suggested that concentrations of 2% or higher in oven-dry herbage are necessary for maximum yields and longevity (68, 114).

Stage of growth has a very pronounced effect on K concentration in alfalfa, often to a greater extent than its

availability in the soil (227). Young plants tend to be high in K, and as the plants mature, the percentage of K decreases (29, 68, 115). With frequent cutting at a young stage, the concentration of K in the plant is found to be higher, thereby increasing the significance of K fertilization. If adequate K is not present, alfalfa stands quickly degenerate to grasses and weeds (151).

Losses of soil K occur due to leaching, erosion and cropping. Potassium availability can also be reduced by excessive rainfall, resulting in a lack of oxygen, which is required for respiration and K uptake (131).

Smith (191) demonstrated that under cool temperatures the K concentration of alfalfa was lower than under warm temperatures, even though the available soil K was high. Consequently, Smith (195) advised that a higher level of exchangeable K in the soil would be required to ensure adequate K in the plant when temperatures are low. Oliver and Barber (160) reported that the efficiency of K uptake appeared to be closely related to the total root area of the plant, while transpiration rate had little effect on rate of K uptake.

Potassium affects a number of plant processes, which include: (i) synthesis and degradation of carbohydrates and translocation of starch resulting in greater leaf area and a delay in leaf senescence; (ii) N metabolism and synthesis of protein, thereby reducing the level of non-protein N; and (iii) promotion of growth of young meristems.

Thus, if K is deficient, N tends to accumulate in the plant as soluble non-protein N and the resulting amino acids are not readily assimilated into protein (214, 248).

Dionne (63) and Klebesadel and Brinsmade (116) reported that the percentage of crude protein in alfalfa was unaffected, or slightly depressed, while dry matter yield and total crude protein production increased with increasing rate of K fertilization.

The rate of K required can be determined by knowing the amount of K in the soil, and how much K the alfalfa crop will remove. The difference is then made up with the addition of K fertilizer (174). Some soils have sufficient K reserves to withstand mining, while others do not. Buker (42) applied 249 lbs of K/acre and removed 520 lbs of K/acre with a dry matter yield of 8.2 tons/acre. Drake et al. (68) demonstrated the increasing importance of K fertilization in the years after establishment, by increasing alfalfa yields 7, 45, 130 and 450%, respectively, the first, second, third and fourth years.

Broadcast applications of a very high rate of K as KCl at establishment time may cause some temporary injury or possible thinning of stand due to a high concentration of chlorine (99, 195). It may thus be necessary to apply the K fertilizer in split applications or to use K_2SO_4. Broadcast applications of K on established alfalfa appear to be effective, and recovery of essentially all topdressed K has been noted (64, 99).

It has been shown that penetration of topdressed K is largely limited to the top few inches of the soil profile except in sandy soils (51, 65). With adequate moisture, the yield of alfalfa may not be affected by lack of movement of K. However, Barber (14) observed that in dry years yields may be reduced considerably since the plant must feed at greater depths where the level of available K may be quite low.

Long-term studies have demonstrated that persistance of alfalfa is largely dependent upon the relative abundance of K (38, 90, 131). At least one annual application of K has been recommended (51, 119), while two or more applications have been advised on sandy soils, especially when the growing season is long and yields are high (178). In northern Florida, Ruelke and Prine (180) reported no significant difference in yields between fertilizer rates the first harvest season, but hay yields and plant persistence declined less the second third harvest season where 2240 and 3360 kg/ha of 0-10-20 (N-P_2O_5-K_2O) was used. Four split applications during the same harvest season were not significantly better than two split applications at the same rate. Significant differences in hay yields in response to split applications of 1120, 2240, and 4480 kg/ha were not obtained until the third season.

Kimbrough et al. (115) noted that yields and leaf area indices of alfalfa increased faster with liberal than with low K. Furthermore, cutting intervals were shortened, there-

by allowing an additional cutting during the season. Accordingly, Barber and Humbert (15) observed that, in theory, an application of K for each cutting would be the most efficient practice.

Accumulation of Carbohydrate Reserves in Alfalfa

The Primary Role of Carbohydrate Reserves

Following their extensive research and review of previous work on alfalfa, Graber et al. (81) reached the following conclusions:

> ...that new top growth, especially in the early stages of alfalfa plants, are initiated and developed largely at the expense of previously accumulated organic reserves; that the roots of alfalfa were the principle organs of storage; that storage occurred principally during the maturity of top growth; and that progressive exhaustion of such reserves by early, frequent and complete removal of top growth resulted ultimately in the death of the plant, regardless of the most favorable climatic and soil environment. (p. 123).

Organic food reserves were defined by Graber et al. (81) and Weinmann (239) as those substances which are elaborated by the plant and stored at certain times in the most permanent organs of the plant body, to be utilized at a later stage by the plant as a source of energy for maintenance, or as building materials for new top and root growth. Graber et al. (81) further found that alfalfa stored most of its food reserves as starch and sucrose. Although the crown is a storage area, the

largest amount of total nonstructural carbohydrates (TNC) is stored in the roots (107, 226). Ueno and Smith (226) found that about 75% of the TNC was in the root and 25% in the crown at all stages of regrowth regardless of plant size.

May (137) and Tan and Baeumer (212) have raised some doubts regarding the specific role of carbohydrate reserves in initiating regrowth, and is determining the extent of regrowth. However, several workers cited by Smith (196), and elsewhere (60, 166, 236) have established beyond doubt that the concentration of soluble carbohydrates in herbage does influence regrowth after cutting. Root TNC decline during regrowth as new top growth is produced and the decline continued for 2 to 3 weeks under field conditions before re-accumulation occurs (83, 154, 187). Smith and Marten (205) found that 40% of the TNC in alfalfa roots had been translocated to the shoots by the time they were 15 cm tall. Smith and Silva (199) found that between the day when tops were removed and the date of lowest carbohydrate level in the roots, 15% of the TNC was used in root respiration, 66% was used in production of new roots and crowns. Ueno and Smith (226) reported that when the minimum level of TNC was reached in the top roots, 63 to 78% was utilized from the roots and 24 to 28% from the crown, regardless of plant size.

Other Functions of Carbohydrate Reserves

Cold resistance

The importance of carbohydrates in relation to cold tolerance was first reported by Steinmetz (209) who established that total sugar concentration was higher in hardened than in non-hardened plants, and higher in tolerant than in sensitive varieties. Graber et al. (81) reported that alfalfa was more susceptible to winter injury when its roots contained low concentrations of non-structural carbohydrates. Several reports in a review by Jung and Larson (106) have also indicated an association between the concentration of sucrose, a protectant, or total soluble sugar, with tolerance.

Heat and drought tolerance

Both cold and drough stresses dessicate cells. Hence, many physiological changes that occur during hardening to drought and cold are similar (126).

Maintenance

Carbohydrates have been found to be the main source of energy for overwintering. Bula and Smith (43) and Graumann et al. (84) found that as much as 50% of the available carbohydrates stored in the roots during autumn were used in respiration during winter. Thus, plants low in carbohydrate reserves are most likely to be injured or killed (189).

Factors Affecting the Accumulation of Carbohydrate Reserves

Frequency and age of cutting

Plants go through periods when the carbohydrate foods are used and when they are stored; a cyclic pattern occurs between early growth and maturity. The cyclic pattern in alfalfa was outlined by Graber et al. (81). With the initiation of growth in the spring, or after cutting, carbohydrates stored in the roots were used to initiate new top growth. Depletion continued until about 15 to 20 cm of top growth was produced. An excess of carbohydrates was produced by photosynthesis in the leaves and stems with 15 to 20 cm of top growth. This excess was then translocated to the roots for storage. Storage of carbohydrates in the roots continued until the highest level was reached near full bloom. There was some removal of carbohydrates from the roots between full bloom and mature seed, as new shoots were initiated from the crown.

The cyclic pattern outlined by Graber et al. (81) has been obtained by other workers (72, 177, 241). The unanimous conclusion has been that, cutting or grazing when food reserves are at a low level may leave very little energy available to start new growth. The greatest damage would be done with too early, too heavy or too frequent cutting of alfalfa plants. Such plants also become more susceptible to drought, heat, winter injury and disease (81, 126, 189).

Weather and soil moisture

Plant development may proceed rapidly with warm temperatures, limited moisture and an abundance of sunshine. On the other hand, it may be prolonged with cool temperatures, abundance of moisture, and cloudy weather (101, 132, 155, 191, 193, 194, 253).

In high temperature geographical areas, investigators have noted an association between high temperatures and low accumulation of carbohydrate reserves due to high respirational losses (176, 177, 241). Increased rates of respiration, the oxidative breakdown of carbohydrates, would be expected since, for each 18F increase in temperature between 50 and 80F, the respiration rate of plants goes up 2 to 2.5 times (138). Therefore, longer intervals between cutting up to advanced stages of bloom have been recommended in order to admit adequate storage of root reserves, and to maintain productivity and persistence.

Height of cutting

The effect of cutting height on alfalfa seems to vary with cultivars, location, season, and frequency of cutting. Beardsley and Anderson (25) reported that alfalfa could be cut closely, but not frequently, from the standpoint of longevity. In an early basic study, Hildebrand and Harrison (92) reported that 15-cm stubble height produced the most recovery growth when harvested weekly or bi-weekly, while 7.5-cm stubble height produced the most when harvested monthly. Other workers have suggested that a tall stubble,

by retaining a photosynthetic residual leaf area, is sometimes desirable for providing additional energy for the initial regrowth after cutting, especially when food reserves are low (40, 120, 198, 251). Langer and Steink (121) also noted that in dry environments where growth rates are slow, the plant would depend on residual leaf area to supply assimilates for a long time.

Robison and Massengale (176) and West and Prine (241) found that decline in herbage yields and stands were less when plants were cut to a 10-cm rather than 2.5-or 5-cm stubble. In the latter study, day temperatures of alfalfa stems at the soil surface were markedly higher in the short clipping height. It was suggested that the lower temperatures in the tall stubble had reduced the respiration rate of the plants, thereby allowing more of the carbohydrates to be conserved.

In other studies (158, 228) increasing stubble height significantly and progressively reduced herbage and protein yield in alfalfa. Leach (123) has pointed out that leaving a stubble to provide more sites for regrowth may be more important than carbohydrate reserve level or residual leaf area. He further suggested that the rapidity with which new leaves are formed after cutting may be more important than the amount of leaf area left on the stubble. As Brown et al. (41) observed, these older and less efficient leaves may be more of a handicap than a benefit to regrowth, especially if they photosynthesize slowly, shade the plant base,

and prevent new shoot development.

Removing and Analyzing Total Nonstructural Carbohydrates

Estimating total nonstructural carbohydrates (TNC) content usually is desired in management studies rather than estimating each individual fraction of the carbohydrates (sugars, starch and fructosans) available as energy to the plant (192, 239). Some knowledge of carbohydrates involved, particularly the non-structural polysaccharide type present, should be available before selecting a method. The common biennial and perennial forage legumes including alfalfa (*Medicago* spp.) are characterised by sucrose and starch accumulation (Smith, 192). Starch is the primary non-structural polysaccharide accumulated in species of the *Leguminosae*. Removal of TNC from these species has been done with acid solutions (87, 201), and with various diastastic enzyme preparations (81, 87, 154, 201, 239). The most used enzyme preparation, takadiastase, hydrolyzes disaccharides and starch to monomers since it contains invertase, maltase and amylase enzymes. In the acid methods, the sugars and non-structural polysaccharides are hydrolysed to monomers by the acid.

Smith et al. (201) reported that 0.2N H_2SO_4 hydrolysis of alfalfa roots gave seasonal TNC trends similar to those obtained from takadiastase. Later, Grotelueschen and Smith (87) reported fructose destruction with 0.2N H_2SO_4, and that xylose was present, indicating hydrolysis of some structural

carbohydrates. No xylose was found in the takadiastase extractions. However, 0.2$\underline{N}$ H_2SO_4 hydrolyzed less than 40% of the starch to glucose in the alfalfa root tissue where starch was present in large amounts (15.5% of the dry weight). Thus, the acid method is apt to over-estimate where little starch is present, and to under-estimate where large amounts of starch are present.

Starch, a glucose polymer, contains two forms, amylose, a linear molecule of low molecular weight, and amylopectin, a highly branched molecule of high molecular weight. Water will not remove all the TNC from tissues containing starch. Amylose which is largely soluble in hot water comprises 10 to 30% of starch, the remaining 70 to 90% being amylopectin which is not soluble in water (Akazawa, 3).

In view of these findings, Smith (192) concluded that the most accurate overall method to remove TNC was one that uses a takadiastase enzyme. This method was first described by Weinmann (239) and later slightly modified by Lindahl et al. (127).

Smith (197) has reviewed the influence of drying and storage conditions on TNC analysis of herbage tissue. He warns that heat drying at high temperatures (above 80C) can cause thermo-chemical degradation, while slow drying at low temperatures (below 50C) allows time for dry matter losses by respiration and enzymatic conversions. The most acceptable heat drying results have been obtained by drying for a short time at a high temperature (i.e.100C), and there-

after at a moderate temperature (i.e., 70C). Smith (197) further noted that changes in TNC concentrations occur during the storage of either freeze- or heat-dried tissues. Consequently, he advised that analyses should be conducted as soon as possible after tissue sampling.

Estimation of Food Reserves by the Growth-in-the-Dark Method

Growth-in-dark studies to provide estimate of the regrowth potential or status of organic food reserves have been carried out with grasses (1, 46, 66), and red clover (111). Plugs of soil containing the plants were dug from the field and placed into plastic pots. The aerial parts of the plants were trimmed off. The pots were placed in dark growth chambers where the plants were allowed to grow and exhaust themselves at constant optimum temperature and sufficient moisture. All new growth was harvested, dried and weighed. TNC were estimated in separate aliquots of plants collected at the same time from the field. In all the above four cases, a positive correlation was obtained between the percentage and actual amounts of reserve carbohydrates present in storage organs and the observed regrowth potential, which was produced in the absence of photosynthesis.

Estimation of Food Reserves by Root Dry Matter

Nielsen and Lysgaard (156) reported that many individual constituents of the root reserves decreased and increased similarly to the total amount of root dry matter. This principle supports a technique recently employed by Wolf (250) as

a guide in telling when to cut alfalfa. By taking weights of roots before and after they were dried in an oven, he showed that when the alfalfa root reached about 28% dry matter, its food reserves were high enough to support vigorous regrowth after harvest, if other conditions were favorable.

The Effect of Cutting Schedules on Alfalfa Productivity

Cutting at Early Maturity vs Late Maturity

The highest yield per acre of nutrients in any one crop of alfalfa has been reported to be at near 10% bloom (196). In these reports, alfalfa harvested at a pre-bud stage of development had a high concentration of feed nutrients but herbage yields at immature stages were low. It was also shown that although the yield of hay continued to increase between one-tenth bloom and full bloom, that it was due largely to an increase in the yield of fiber. The yield of TDN, protein and minerals generally decreased after early bloom principally because of the loss of the lower leaves due to age, disease, lodging, etc. 175, 246).

Fuess and Tesar (76) obtained higher herbage yields from three cuttings per year at near early bloom than from two cuttings at near full bloom, primarily due to less leaf loss. Leaf losses accounted for two-thirds of the difference was due mainly to higher rates of net photosynthesis in the younger leaves of the 3-cut alfalfa.

Time Interval Cutting

Smith (196) has cited a long list of workers who have investigated the effects of cutting based on fixed cutting intervals or number of cuttings per year. In general, 35- to 42-day intervals between harvests have provided the best results in seasonal herbage and nutrient yields per hectare and in stand persistence. However, the most satisfactory time interval between harvests will vary with location and season of the year, since speed of plant development is affected markedly by temperature and soil moisture conditions (Wilsie, 247).

Stage of Growth Cutting

In a survey ofthe literature, Smith (196) cited numerous workers who have studied the effects of cutting alfalfa at definite stages of development in contrast to time interval or calendar-date cutting. The general observation has been that although calendar-date cutting is easier to follow, harvests will occur at varying stages of development since growth rates do vary with environmental conditions. There is also the chance of harvesting at an immature stage that may markedly reduce plant vigor (Smith et al., 203).

Plant maturity on a particular date varies among years and locations (247). Therefore, cutting based on fixed intervals between cuttings or on calendar dates would not take into consideration the physiological stage of development of the plants. During a high temperature period

alfalfa has been seen to flower in less time than at lower temperatures (101, 132, 155, 191, 193, 194). Cultivars also differ in rates of development (135, 202).

Using a growth chamber, Marten (132) has shown that the chances of repeatable quality are better if a flowering stage rather than a chronological age criterion for harvest is used. In a review article on the subject, Keoghan (112) concluded that varying cutting dates according to rate of growth during the season was much better than using pre-determined dates, and that consistent herbage quality could be obtained only by harvesting at a consistent stage of development.

Most cutting investigations based on stage of development showed that harvesting at the first flower stage was the best compromise for acceptable herbage and nutrient yields and stand persistence in the northern United States (190, 202), the southwestern United States (72) and the Valley of Mexico (44). At this stage, root reserves were found to be at reasonably high level, and crop growth rate had begun to decrease markedly. Nelson and Smith (154) and Smith (190) reported that accumulation of dry matter decreased rapidly just prior to the appearance of the first flowers, and thereafter the increase in weight of tops was due largely to the fibrous fractions from the elongation and enlargement of stem internodes.

Some investigations in high-temperature areas have shown that alfalfa should be allowed to go to advanced stages

of bloom during the summer months in order to permit adequate storage of root reserves in excess of the high respirational losses, and to maintain productivity and persistence (176, 241). Crowder et al. (59) reported that at high altitudes near the equator, flowering was sparse and sporadic. In other areas growth has continued during mild winters without flowering (61). Under these conditions flowering could not be used as a guide to cutting. Crowder et al. (59) found that cutting when the new shoots developed at the crown was the only reasonable guide to indicate when to cut. According to Willard (244) and Willard et al. (245), shoots begin to grow either when the level of carbohydrate root reserves is high, or when prolonged drought is broken by rain, or when the shoots lodge to expose the crown to more light.

Cutting Based on Heat Units

The heat unit approach has been used in studying successive stages of plant growth and temperature relationships by the accumulation of daily mean temperatures above a certain threshold or base temperature during the growing season (235). The sum required for a particular crop variety has been assumed by heat unit workers to be a constant value, i.e., that a given stage in the development of any plant is reached when that plant has received a certain amount of heat, regardless of the time required.

The base temperature or zero point of vital activity has been determined by experimental methods and differs in different crops, the early sown crops having a lower base temperature than the late sown (11). Arnold (11) used a regression equation from temperature and the rate of development, and solved the equation for temperature when the rate of development was zero.

The main disadvantage of the heat unit system were summed up by Wang (235) and Wilsie (247). They observed that plants do respond differently to the same environmental factors during various stages of their development; yet, varietal constants obtained from heat units computations fail to take into account these time sequences. Secondly, the threshold temperature, which is considered as a constant, is applied to the entire life cycle of the plant. Yet, the threshold values change with the advancing age of the plant. Moreover, the upper threshold is not taken into account. Thirdly, growth and development are not directly proportional to temperature, especially to supraoptimal values. Also, these systems provide no consideration for the effect of alternating day and night temperature and the diurnal range. Fourthly, the heat system does not take into account many factors which influence plant growth and development, such as soil type, fertility, moisture, topography, altitude and latitude. Frost and drought damage, wind, hail, storms, insects and disease may influence the heat unit values necessary to bring a crop to a given stage of maturity.

Present Outlook

Data obtained in the northern United States (196) and Canada (225) have shown that the availability of cultivars with high persistence has made it more feasible to adapt cutting schedules to obtain high seasonal yields of feed nutrients. Consequently, Smith (196) believes that more emphasis could now be placed on cutting at early maturity to obtain high nutrient yields, with less emphasis on schedules to maintain stands.

The Nutritive Value of Forages

Introduction

Nutritive value as described by Mott (146) and Mott and Moore (147) consists of the chemical composition, digestibility of the components and the nature of the digested fractions. Raymond (168) stated that the nutritive value of a forage should be considered as composed of a complex of parameters that determine the nutrient intake of ruminant animals fed on that forage. He expressed nutrient intake as equal to: intake of feed x digestibility of feed x efficiency of utilization of digested feed, each of which should be investigated separately before their interactions in practical systems of ruminant feeding are considered. The importance of this approach was indicated by Ingalls et al. (98), Reid (170) and others (47, 58, 207) who demonstrated that intake was

the more important factor than digestibility when comparing the nutritive value of various forages.

Barnes and Gordon (19) (using 'feeding value' and 'nutritive value' synonymously) have stressed the importance of distinguishing between potential feeding value and attained levels of animal production, which are influenced by non-forage factors, such as ration ingredients, animal potentials, feeding environment and interactions of these factors. The scope of this review will be limited to those factors that influence the potential feeding value of forages, in general, and of alfalfa, in particular.

Regulation of Forage Intake and Digestibility in Ruminants

The development of some of the current concepts on voluntary intake have been reviewed by Balch and Campling (11), Conrad (54) and Brown (39). In brief, intake by non-ruminants is controlled mainly by levels of blood metabolites, the animal ceasing to eat when these reach a threshold level. In ruminants, intake depends much more on the capacity of the rumen. Eating ceases when a certain degree of fill has been reached, and starts again when fill has been reduced by digestion and movement of food residues through the digestive tract. Only on feeds of high energy concentration does blood metabolite level, rather than gastro-intestinal fill begin to control the amount of food that ruminants will eat (54).

Ruminants are able to eat more of highly digestible forages than of less digestible forages because the latter occupy more volume and are within the rumen for a longer time, and because from them more indigestible residue has to be passed down the hind tract (12).

A decrease in voluntary intake as forages become more mature, and so less digestible, has been shown in numerous experiments. However, while with most forage species intake decreases as the forage becomes less digestible, the relationship between intake and digestibility can differ markedly between different forages. Thus, Osbourn (161) and Osbourn et al. (162) showed a higher level of intake of alfalfa than ryegrass and timothy at a given level of digestibility. Van Soest (230) reported that alfalfa contained a higher proportion of cell contents and a lower proportion of cell wall constituents than grass of the same level of digestibility. The digestible fraction of alfalfa could thus occupy less volume and time within the rumen; as a result, the animal would eat more of it than the grass.

There is the possibility that rate of digestion, and, in turn, the rate of intake, may be affected by conditions within the rumen. Tilley et al. (219) showed that the rate and extent of cellulose digestion decreased as the pH of an *in vitro* system (and by analogy of the rumen *in vivo*) diverged from the physiologically normal level of about

pH 6.8. Some forages, particularly highly buffered, low sugar, forages such as the legumes, are found to give a characteristically higher rumen pH (6.6 to 6.8) than grasses. Raymond (168) suggested that these differences in rumen pH could lead to a higher rate of digestion of the cell wall fraction of alfalfa than ryegrass, and that this could partly account for the higher level of intake of alfalfa and of other legumes.

Reid (170) demonstrated that intake was the more important factor than digestibility when comparing the nutritive value of various forages. While energy digestibility (TDN) increased by only 36% when changing from lowest to highest quality forage, intake increased by 250%. In terms of animal production, other workers (47, 58, 207) showed that voluntary intake may account for about two-thirds of the variability in animal performance, while digestibility may account for about one-third.

Blaxter and Wilson (30) found a curvilinear relationship, intake increasing less rapidly with digestibility at higher levels of forage digestibility. Hutton (97) and Conrad et al. (56) found an increase in intake above a forage dry matter digestibility of about 70%. Baumgardt (22) found that the intake of a roughage concentrate mixture did not increase when the energy digestibility was above 67%. The results of Hutton (97) and Conrad et al. (56) suggest that at high levels of forage digestibility, intake begins to be limited by metabolic factors, namely,

blood levels of organic acids, glucose, etc., rather than by gastro-intestinal fill. That is, in intake terms, the ruminant begins to behave like a non-ruminant (54). Van Soest (230) suggested that this is likely to occur when forage dry matter contains less than 55 to 60% of cell wall constituents.

However, the level at which forage digestibility no longer limits intake must, to some extent, depend on the physiological condition of the animal being fed, that is, on the critical level of blood metabolites at which it ceases to eat. Osbourn et al. (162) found no divergence from linearity with a wide range of grasses and legumes up to 80% dry matter digestibility. They used thin sheep, with a high growth potential, which may therefore have had a higher threshold intake level than, say the mature sheep used by Blaxter et al. (32).

The Effect of Supplementary Feeds on Forage Intake

Evidently, in most cases, supplementary feeds do partly replace rather than supplement the forage with which they are fed. As increasing amounts of supplementary concentrates are fed, the ruminant animal eats less forage (31, 49). This decrease in forage intake appears to be more marked with forages of high digestibility, i.e., of initially high intake, than with forages of lower digestibility and intake. Such forages tend to behave like concentrated feeds, so that their voluntary intake may be determined by levels of blood

metabolites, rather than by rumen fill. In this case, level of forage intake might be expected to decrease as a result of the enhanced levels of blood metabolites following concentrate feeding.

The reduction in intake of forages of lower digestibility may in part be related to the effects of supplementary feeding on rumen pH. As the level of concentrate feeding increases, rumen pH tends to decrease (37, 222), and it is possible that this causes a reduced rate of digestion of the cell wall constituents in the forage (219), and so reduced level of voluntary intake.

Prediction of Forage Digestibility from Chemical Composition

For nearly a century the extent of forage digestion was predicted from its proximate analysis based on Weende crude fiber, crude protein and nitrogen-free extractives. Sullivan (210) and Dijkstra (62) both showed that the relationship between digestibility and chemical composition become less precise when these analyses are applied to a wide range of forages. Forages become less digestible as they become more fibrous with advancing maturity. However, at a given fiber content, different forages can have markedly different levels of digestibility (102).

An alternative approach using chemical techniques more relevant to the digestion process has been described by Van Soest (231). He considered forages to contain two main fractions, the cell contents which are almost completely digested, and the cell wall constituents, which are only

partly digested by the ruminant. A forage sample is separated into a cell contents fraction soluble in neutral detergent and an insoluble cell wall fraction (neutral detergent fiber), as well as a fiber fraction insoluble in acid detergent (acid detergent fiber) and lignin. Van Soest and Jones (232) were then able to compute the apparent digestiblity of forages using an equation that took into account the cell contents, the cell wall contents, the acid detergent fraction, silica content and the endogenous materials which result from the digestion process and are lost in feces.

Estimation of Forage Digestibility by *In Vitro* Techniques

The *in vitro* digestion procedure developed by Terry and Tilley (216) attempts to simulate the biological process of digestion. A two stage procedure comprising digestion by rumen microorganisms in an artificial rumen followed by digestion by acid-pepsin has given the closest agreement with *in vivo* digestibility values for the dry matter and organic contents in forages. This method showed a correlation coefficient of 0.97 between *in vitro* and *in vivo* values when tested on a wide range of grasses and legumes, and the following equation:

Digestion *in vivo* = 0.99 x digestibility *in vitro* - 1.01. Bosman (35), Oh et al. (159), and Ademosum (2) have also reported higher correlations between *in vitro* and *in vivo* methods than the chemical methods tested.

Raymond (168) pointed out that when interpreting the error terms of the relationship between *in vitro* and *in vivo* values it is important to realize that errors do not arise solely from deficiencies in the laboratory techniques, but also from the actual measurement of the *in vivo* digestibility of the forages. More importantly, digestibility *in vivo* is not a constant parameter of a particular forage. Thus, digestibility determined in an animal experiment may depend on the amount of forage fed, digestibility decreasing as the level of feeding increases (142), and can be significantly reduced if the animal is parasitized with stomach worms (185, 208). Accordingly, Raymond (168) concluded that the main area for improvement lay in the better standardization of the *in vivo* digestibility experiments, the preparation of the forage samples for analysis, and the conduct of the laboratory procedures. Barnes (17) also emphasized the importance of the general adoption of the standardized *in vitro* digestibility procedures so that results reported by different laboratories may be directly comparable.

It has been stressed that the system should wherever possible be checked with relevant samples of known *in vivo* digestibility. Thus, Raymond and Terry (169) reported low *in vitro* digestibility levels when both the test forage and the feed of the donor animal were of low nitrogen content. The higher level of digestibility *in vivo* resulted from the animal's ability to re-cycle urea via salivary and ruminal

secretions, whereas digestibility *in vivo* was limited by a deficiency of nitrogen in the combined sample and inoculum. Addition of some N as urea to the *in vitro* system later increased sample digestibility to the *in vivo* level. However, care must be taken since a depression in *in vitro* digestion (70) or no increase in forage intake (109) have resulted from N addition to a sample or forage already of high protein content.

Minson (140) reported that the rate of digestion within the rumen, and so level of intake, of forages of very low crude protein may be limited by the lack of nitrogenous substrate for the rumen microorganisms. The critical level of feed protein depends on the type of forage, but it is commonly in the range 4 to 6% crude protein (140). Minson and Milford (141) increased the intake of Pangola digitgrass (3.6% CP) when a supplement of alfalfa was fed, to a maximum intake at a total diet content of 6% CP, after which the intake of the grass decreased as it was progressively replaced by alfalfa.

General Characteristics of Alfalfa Feeding Value

The nutrient content of alfalfa is well known (157). It is especially high in protein and Ca, and is a good source of both macro and micro nutrients needed by the rumen organisms for normal rumen function (26, 104, 122).

The *ad libitum* intake of digestible nutrients of alfalfa by livestock is greater than for most other forage species since much of the dry matter of alfalfa is in a non-fibrous

form that is readily available for absorption in the ruminant digestive system (172, 229). Although the levels of the fibrous material in alfalfa are relatively low in comparison to grasses, it is highly lignified and generally less available than the fibrous material from grass species (103, 211, 221). Smith et al. (206) suggested that lignin limits the extent of digestion but has comparatively little influence on the rate of digestion. They reported that rates of cell wall digestion of alfalfa and grasses were most highly correlated with the cell soluble content of forage, although cellulose and hemicellulose were more casually related to rates of cell wall digestion than are the levels of cell solubles.

Balwani et al. (13), Barnes and Mott (21), and Troelsen and Campbell (223) compared alfalfa with grasses at essentially the same level of dry matter digestibility. Despite equal digestibility coefficients, the intake of grass and the daily gain achieved from it were lower. This is because more digestible nutrients from grass must be derived from the digestion of fiber, which is a slower process than absorption of soluble nutrients. Therefore, intake, passage and digestion of alfalfa proceed at a faster rate, and the animal consumes more digestible nutrients per day from the alfalfa even though both rations are of the same digestibility.

It has also been suggested that the digestible energy obtained from the more fibrous materials is not as effi-

ciently used for fattening as that obtained from material of higher quality (128, 145). The total advantage of high quality alfalfa therefore goes beyond that indicated by digestible nutrient content, and is compounded by a potential for being consumed at higher levels, a faster rate of digestibility, and perhaps a more efficient conversion of digested energy to productive energy.

Forage Factors Influencing Alfalfa Feeding Value

Growth stage

The major factor affecting the feeding value of alfalfa, as well as other legumes and grasses, is the reduction in digestibility and rate of digestion reflected in lowered voluntary intake with advancing maturity (55). Van Riper and Smith (227), Blaser (28) and Reid et al. (171) presented data on the effect of advancing maturity on the chemical composition of forages. Numerous observations have been reported on higher digestibility of immature alfalfa with greater voluntary intake and animal performance potential than mature alfalfa (19). Likewise, there are several reports on the decrease of protein content and increase in fiber and lignin with advancing maturity of alfalfa (19, 196).

As the date of cutting is delayed, yield generally increases with an increase in the amount of stems, a lowering of the leaf to stem ratio, and a change in the chemical composition (57). These workers emphasized that the restriction on nutrients caused by maturation of alfalfa induced or

increased the severity of deficiencies of energy, protein, Ca and P. Further, Conrad et al. (57) reported that declining digestibility resulting from advancing maturity reduced milk production partly because of a lowered concentration of digestible energy in the forage, and partly because of lowered voluntary intake.

Troelsen and Campbell (223) found a curvilinear relationship between voluntary intake of digestible organic matter by sheep and the time of alfalfa harvest. A delay of one day in harvest time caused an average daily reduction in voluntary intake of alfalfa hay of 0.29 g/kg $BW^{.75}$. Anderson et al. (8) reported a decline of 0.21 g/kg $BW^{.75}$ in dry matter intake, 0.28% digestibility and 0.20% crude protein, for each day of delay.

Initial vs aftermath cuttings

First cutting alfalfa has been characterized by a larger decline in digestibility with increasing maturity, compared to subsequent cuttings (23, 95, 171). In general, later cuttings are not as high in feedingvalue at the early stages and not as low at the later stages as the first cuttings (71, 95, 171, 184, 185). However, Jung et al. (108) found that dry matter digestibility of second and third cuttings of alfalfa was similar to the digestibility of the first cutting when harvested at a comparable stage of growth.

Lowe and Reid (129) found that lambs consumed 7% more dry matter (DM) and digested 6% more total DM from the three-cut than the two-cut management system, measured over a 2-year

period. The animal productivity value (daily digestible DM intake) for the three-cut system was 27% greater than for the two-cut system.

Recommendations for optimum harvest schedule have varied over a range of growth conditions and include mid-bud, first flower, 10% bloom, appearance of new basal shoots, and the falling of lower leaves (196). Although the highest quality feed is produced from immature alfalfa, a consideration of yield, stand survival, and weather conditions that are conducive to conservation may indicate that greater returns are realized at a more mature stage.

Environmental conditions fluctuate seasonally and yearly and from location to location so that different lengths of time may be required for a plant to reach a certain stage of growth (203, 247). Thus, harvesting by calendar date generally has not been a reliable an indicator of forage feeding value as growth stage. In livestock feeding programs, it is desirable to have forages that have a minimum variation in feeding value from harvest to harvest to reduce alterations in rations. The variability of alfalfa has been shown to increase when harvested on a calendar basis, as compared to harvesting according to stage of growth (139, 249).

Climatic factors

Harvesting on specific calendar dates at widely separated geographic locations, e.g., different latitudes, resulted in alfalfa being harvested at several maturities

(105, 203). Different elevations also resulted in maturity differences similar to shifts in latitude (7). On the same date, alfalfa was most mature at the lower elevations, but the rate of daily decline in DMD was greater at the higher elevation.

Similarly, seasonal and annual variations in environmental factors such as day length and light intensity, as well as moisture and temperature, do influence chemical composition and estimates of feeding value (23, 53, 77, 188).

Studies under controlled environmental conditions (77,85, 101, 132) confirm conclusions from field studies that the primary effect of high temperature is hastened maturity and a concomitant decline in quality. Alfalfa harvested at specific growth stages under warm vs cool temperature regimes was higher in percentage of crude protein (132, 191, 233), and leaf percentage (132, 200). However, warm temperatures decreased IVDMD (191, 223), nonstructural carbohydrates (132, 191, 200) and dry matter yield (191, 233).

Soil type

Meyer and Jones (139) reported that lignin was lowest and protein highest in alfalfa produced on clay loam soils, as compared to sandy soils. These patterns might have been due to a higher leaf to stem ratio and short plant height for alfalfa grown on clay soils, as compared to loam or sandy soils (254). Compositional differences noted on soil types at widely separated locations are difficult to

evaluate because of confounding with other environmental factors (124).

Soil moisture and temperature

Soil moisture and soil temperature have had variable effects on the percentage of crude protein content of alfalfa (36, 79, 86, 132, 233). However, reduced yield brought about by drought may result in a stunted, leafier plant, which is lower in fiber and lignin and more digestible (101, 233). On the other hand, too much soil moisture may reduce yields and lower the percentage of nutrients. With irrigation at a 50% level of minimum available soil moisture, Bezeau and Sonmor (27) obtained higher dry matter yields of alfalfa, and a higher percentage of crude protein and *in vitro* digestible cellulose than either higher or lower levels of irrigation.

Soil fertility

The composition and nutritional value of alfalfa is affected by the availability of several chemical elements in the soil (173). From a 10-year study, Hanson and MacGregor (90) concluded that the concentration of P and K in a 10-year-old alfalfa was related to the quantities of these elements applied by annual topdressings. Smith and Albrecht (204) reported that excessive use of a single fertilizer element, or unbalanced fertilization, resulted in maximum yield but lower biological value. Wedin et al. (238) reported increased daily gains with moderate fertili-

zation of P and K (182 kg/ha), whereas heavier rates (363 kg/ha tended to decrease weight gains. Calder and MacLeod (48) found that the *in vitro* digestibility of first cut, but not second cut, alfalfa was increased by K fertilization. Fertilization may also increase the yield of nutrients per unit area, through an increase in dry matter yield with no influence on digestibility (237).

Composition of leaves and stems

Several reports have shown that leafiness in alfalfa is associated with quality, since leaves contain more nutrients than stems (19, 196). The percentage of leaf tissue declines as the number of days between harvests is increased (84, 101, 113), and with maturity (108, 130, 148, 254). Leaves are also more uniform in protein and fiber percentages throughout the growth period (130). Smith (191) found that all chemical constituents were in higher concentration in the leaves except for sugars, crude fiber and K.

Other reports indicate that stems contain approximately 75% of the cellulose or crude fiber of the plant (108, 254), and that the lignin percentage of the stems is often more than three times that of leaves (132, 148). The lignin percentage of leaves remains constant with maturation (217), and lignification is considered of little importance in leaves (130). This view is supported by nearly constant level of digestibility of total leaf tissue observed at various stages of maturation (73, 217).

Digestibility and intake of leaves and stems

The *in vitro* digestibility of alfalfa stems was reported to decrease steadily during maturation, while the digestibility of leaves changed only slightly (73, 130, 149, 217). Mowat et al. (149) and Terry and Tilley (217) found that alfalfa stems were never as digestible as the leaves in the early growth stages, although Barnes and Mott (20) found that stem tissue in the upper part of the plant was more digestible initially than the leaf tissue.

Troelsen and Campbell (224) reported that leafiness accounted for 85% of the variability in intake by sheep of digestible organic matter in alfalfa hay. They found that the effect of leafiness upon intake was less at later growth stages of the plant, indicating that as leafiness decreased, and conversely as maturity increased, other factors became more important in the regulation of intake and digestibility.

Differences in quality, mainly arising from leaf and stem proportions, occur between top and bottom portions of alfalfa plants (193), the lower part of alfalfa plant being less digestible than the top portion (20, 91, 217). Hence, Troelsen and Campbell (224) emphasized that although leafiness alone may not be adequate as a sole criterion of nutritional quality of alfalfa, quality estimates may well be improved by placing more emphasis on the leaf to stem ratio.

Insects and diseases

The effect of insects and disease on alfalfa yields and stand has been covered by Graham et al. (82) and App and

Manglitz (10). Reduction in the leaf to stem ratio, increase in the fiber content, or reduction in the protein or carotene content by diseases, insects and other pests, invariably lower the feeding value of alfalfa (74, 110, 136, 234).

Varietal differences

Little or no difference has been found in chemical composition and *in vivo* or *in vitro* digestibility among alfalfa varieties harvested at the same stage of growth (6, 20, 108, 135). However, the tendency exists for the later maturing varieties to have a higher digestibility on a given date (149). Secondly, differences among varieties in susceptibility to insect and disease damage can influence leaf loss (243).

The form in which alfalfa is fed

In the United States hay, silage, and greenchop account for more than 95% of harvested alfalfa fed on the farm (19). Feeding value cannot improve after the plant is cut. The preparation of alfalfa hay, silage, greenchop, etc. and their comparative feeding values have been reported by Gordon et al. (80) and Barnes and Gordon (155).

CHAPTER III

EXPERIMENT I

EFFECTS OF DEFOLIATION AT TWO DIFFERENT STAGES OF GROWTH ON HERBAGE YIELD, STAND PERSISTENCE AND LEVEL OF CARBOHYDRATE RESERVES IN AFRICAN AND FLORIDA 66 ALFALFA

Materials and Methods

Two varieties of alfalfa, African and Florida 66, were seeded on October 12, 1971 at the Agronomy Farm, Gainesville, Florida. Planting was made in 25.4-cm rows using a planter at a seeding rate of 22.4 kg/ha. Prior to seeding, 1120 kg/ha of dolomitic lime had been applied on September 13, 1971, to correct the pH of the soil, an Arredondo loamy fine sand (a Grossarenic Paleudult sub-group). Just before seeding, 1120 kg/ha of 0-10-20 ($N-P_2O_5-K_2O$) fertilizer, 11.2 kg/ha of FTE 503 and 22.4 kg/ha of borax were disked into the top soil of the seedbed. Subsequently, 560 kg/ha of 0-10-20 containing 170, 1077, 391 and 412 g of Cu, Fe, Zn and Mn, respectively, were applied to all plots every 6 weeks.

A split plot experimental design was used, with the two varieties of alfalfa being the main plots and the two stages of growth, the subplots. There were six replications. Each subplot (13.7 m long) was made of 14 rows of alfalfa. Herbage samples for estimating dry matter yield and chemical composition were cut at 3 and 6 weeks of growth from the first seven rows, while root samples for percent carbohy-

drate analysis and cores for the regorwth-in-the-dark were taken weekly from the adjoining seven rows.

Dry Matter Yields

For dry matter yield and chemical composition determinations, 5.31 m of the three middle rows of the first seven rows were cut using a sickle-bar plot mower. Total net weight yields were recorded and subsamples of about 0.5 kg weed-free forage taken from each replication and dried at 60C in forced air driers. Their weights were used in calculating total dry matter yields in tons of oven-dried, weed-free hay per hectare.

Percent Carbohydrates in the Roots

From April 20 to November 14, 1972, root samples were collected each week from the adjoining seven rows of alfalfa in each plot. Portions of the roots, approximately 15 cm below the crown, were washed and dried in an electric oven at 100C for 1 hour to reduce enzymatic activity (110), and thereafter at between 70C and 80C for 2 days. The dried roots were ground in a Wiley mill with a 1-mm screen, and stored in sealed polyethylene bags until analysis. Total nonstructural carbohydrates (TNC) were determined by the takadiastase method of Smith (110), and expressed as percentages of root dry matter.

Extent of Branching in Alfalfa Roots

Dried root samples collected every sixth week were

separated into branch roots and the tap root. Their separate weights were used in determining the extent of branching in the two cultivars of alfalfa cut at 3 and 6 weeks in 1972 and at 6 weeks in 1973, by computing the branch roots to total roots ratios.

Regrowth Potential in a Dark Chamber

At the same time of collecting root samples for TNC analysis plugs approximately 15 cm in diameter containing a row of alfalfa plants were dug from the field and placed into plastic pots. The aerial parts of the plants were trimmed off. The pots were placed in a dark chamber where the plants were allowed to grow at a constant temperature of 25.5C and sufficient moisture. All the new growth was harvested after 12 days, dried and weighed. Correlations were made between these weights and percentage of TNC in separate aliquots of roots collected at the same time.

Persistence of Stands

Persistence of stands was determined by staking a 1-m length of one row in each plot. Number of surviving plants were counted in these same locations in the spring and fall of 1972 and 1973.

Statistical Analysis

Analysis of variance of a split-plot design was carried out according to LeClerg et al. (124) on all the data collected. The number of surviving plants at the end of each season

were expressed as percentages of the original stand and transformed to arcsin values before the analysis of variance. Differences between treatments were declared significant at the .1, 1 or 5% levels of significance. Duncan's new multiple range test (69) was used in mean separation at the 5% level of significance. A t-test of paired samples was used in the second season to analyze data collected from African and Florida 66 both of which were cut at intervals of six weeks from adjacent plots.

Results and Discussion

Results of herbage dry matter yields, carbohydrate reserves, morphological characteristics and persistence of African and Florida 66 alfalfa were obtained over a period of two years, while regrowth potential was determined only during the first harvest season.

The first growth of African and Florida 66 alfalfa which was seeded on October 12, 1971, was cut January 19, 1972, following the below freezing temperatures of January 16 and 17 which left most of the top plant parts dead. The dry weight of herbage removed averaged 1.5 and 1.8 MT/ha in African and Florida 66, respectively. The next cutting was made at staging on April 18, 1972, at which stage both cultivars were in mid-bloom. At this cutting, African and Florida 66 averaged 2.3 and 2.8 MT/ha of dry matter, respectively.

The last cutting in the first season was made on November 14. Subsequent regrowth was cut on January 16, 1973 after frost damage. The next cutting was made at the second staging on April 2, 1973. African and Florida 66 cut at the 6-week stage of growth averaged dry matter yields of 1.2 and 1.4 MT/ha, respectively. No further cuts were made at the 3-week stage of growth in both cultivars in 1973

as the 1972 cutting had left plots virtually void of plants.

Dry matter yields of frost-kill and staging cuts were not included in treatment response analyses.

Dry Matter Yield

The seasonal and total dry matter yields of African and Florida 66 at two stages of growth after staging are presented in Table 1 for the 1972 growing season. A total of 10 and 5 cuts were made from the 3- and 6-weekly cut alfalfa, respectively. At the 6-week stage of maturity, Florida 66 produced a total of 8.9 MT/ha of dry matter, which was significantly greater than the 7.7 MT/ha produced by African. Similarly, at the 3-week stage, Florida 66 produced significantly more dry matter (4.7 MT/ha) than African (4.0 MT/ha).

Dry matter yields tended to decline with each consecutive cutting at both stages of maturity, with Florida 66 declining less than African. By the 9th cut, the 3-weekly cut alfalfa produced virtually no yield. Therefore, in the 1973 season yield data were obtained from the 6-weekly cut plots only.

Seasonal and total dry matter yields for 1973 are also shown in Table 1. Florida 66 produced a total of 8.2 MT/ha which was again significantly greater than the 6.2 MT/ha produced by African. After the second cut, dry matter yields declined as the season progressed.

Under these cutting intervals, Florida 66 alfalfa was capable of producing a higher yield over a two-year period

than African. These results further substantiate those which were reported by Prine (167). Under different cutting management treatments, Ruelke and Prine (180) obtained satisfactory yields of Florida 66 over a period of 3 years.

Carbohydrate Reserves

The results of this study are presented in Table 2 and illustrated in Figures 1 and 2, as percentages of TNC in the oven-dry roots of 3- and 6-weekly cut African and Florida 66 alfalfa. Root samples were collected weekly for 30 weeks between April 20 and November 14, 1972. The two alfalfas were cut 10 times at the 3-week stage, and 5 times at the 6-week stage of maturity.

At staging, TNC in the roots were essentially the same in both cultivars. Following each defoliation, TNC declined, and then built up to another peak before the next defoliation. This trend was repeated in both alfalfas at each stage of maturity throughout the season.

At the 6-week stage of maturity, TNC reached the lowest levels approximately three weeks following defoliation (Figure 2). At the first cutting (May 30), TNC in both cultivars had apparently reached their highest peaks and had started to decline. This happened again in Florida 66 at the 2nd cutting (July 11) and in African at the 3rd (August 22) and 5th (November 14) cuttings. Graber et al. (81) and Willard (244) and Willard et al. (245), reported that similar drops in the level of root TNC occurred in mature plants of alfalfa due to

the removal of stored carbohydrates by new shoots being initiated from the crown. In the 3-weekly cut alfalfa (Figure 2), TNC reached their lowest levels sometimes in one week, but mostly in two weeks, following defoliation.

At the 6-week stage of growth TNC in African ranged from 24.4 to 36.9, with a mean of 31.6%. The range for Florida 66 was from 29.1 to 42.0, with a mean of 34.5%, which was significantly higher than that of African. Florida 66 TNC peaks were also higher than the corresponding peaks of African, in all cases. Following each defoliation TNC levels in African dropped more sharply than those in Florida 66. The lowest TNC levels in Florida 66 were much higher than the corresponding lowest TNC levels in African throughout the season.

At the 3-week cutting interval, TNC ranged from 12.2 to 35.1 with a mean of 20.2% in African, and from 14.8 to 38.5 with a mean of 22.1% in Florida 66. These two means were not significantly different at $P < .05$.

At the 6-week stage of growth, out of 5 TNC peaks, four in Florida 66 and only one in African, attained greater TNC percentages than their respective levels recorded at staging. At the 3-week stage of maturity TNC content declined progressively until August when there was a slight upward shift, but neither African nor Florida 66 again reached its TNC level recorded at staging. In most cases, especially from July onward, levels of TNC in Florida 66 remained higher than

those of African. However, the stand of alfalfa cut every 3 weeks grew poorer as the season progressed. The slight increase in TNC after mid-August was probably due to the fact that in the more open stand, the few surviving plants were more adapted, had less competition and therefore could store more carbohydrate reserves. Also, in September, the high temperatures of the summer started dropping.

Previously in Florida, West and Prine (164) had reported that weekly %TNC in Hairy Peruvian alfalfa cut at the first flower stage of development declined progressively to the lowest level in October, and never again reached the initial level recorded at staging. This would indicate that the first flower stage of maturity which averages up to 4 weeks in Florida was, like the 3-week cutting interval in this experiment, not long enough to allow for full replenishment of carbohydrate reserves.

Percentages of TNC in root samples of African and Florida 66 alfalfa at 6 weeks of maturity from November 14, 1972 to October 29, 1973 are presented in Table 3, and illustrated in Figure 3. Percent TNC dropped to their lowest levels in winter and then built up to higher levels by April 2, 1973. In the northern states where winters are colder and longer than in Florida, it has been reported that as much as 50% of the available carbohydrates stored in the roots during autumn were used in respiration (43, 84). The low levels of TNC obtained during Florida's mild winter indicate that

stored carbohydrates were utilized by the alfalfa plants in respiration and maintencance during the season of little or no growth. After April 2, Florida 66 maintained a comparatively even level of TNC (31 to 33%), while African fluctuated between 26 and 31%. Florida 66 had higher %TNC at each sampling time, and an overall significantly higher ($t_{.05}$) mean.

Regrowth Potential

Weekly dry matter yields of dark chamber regrowth of African and Florida 66 alfalfa cut at 3 and 6 weeks of growth are shown in Table 2. These are illustrated together with percent root TNC in Figures 4, 5, 6 and 7. In all cases, the dry weight of regrowth produced in the dark chamber followed a similar trend or pattern as weekly %TNC throughout the growing season. Regrowth weights declined following each defoliation, and then built up to another peak before the next defoliation.

At the 6- and 3-week cutting intervals, African and Florida 66 produced a mean total of 26.8 and 29.9 g and 9.3 and 9.7 g of oven-dry regrowth, respectively. Thus, Florida 66 produced more dark chamber regrowth than African at the same stage of cutting interval, but, unlike %TNC these differences were not statistically significant at $P < .05$.

Regression equations for the prediction of TNC using the dry wight of dark chamber regrowth are shown in Table 4.

All equations show quite high positive and significant ($P < .001$) correlation between dry matter yields of 12-day dark chamber regrowth and percent root TNC. However, in both cultivars, the alfalfa cut at 3 weeks of maturity had regression equations with a lower Y-intercept, higher slope, higher correlation coefficient and higher F value than the corresponding values from the 6-weekly cut alfalfa. Similarly, the pooled regression of African and Florida 66 cut at 3 weeks of maturity had a lower Y-intercept, higher slope, r and F values than those of the 6-weekly cut alfalfa. The mean standard error of estimate ($S_{y.x}$) of the six equations was 2.92.

Similar findings have been reported in grasses (1, 46, 66) and in red clover (111) in which positive correlations were obtained between regrowth potential and the level of reserve carbohydrates. All the dark chamber regrowths in this experiment were harvested after 12-days of growth. At this stage most plants had reached their maximum heights and size and were beginning to lodge (Figure 8). Preliminary studies prior to the experiment had also indicated that regrowths beyond the 12th day were erratic and only a small fraction of the initial regrowth. Furthermore, since regrowth potential was being examined, the 12-day regrowth would be more related to that fraction of carbohydrate reserves that is utilized in the initiation of regrowth in alfalfa immediately following defoliation.

Branch- and Tap-root Proportions

Working in Florida, Prine (167) reported that the roots of most alfalfa plants died up to within 15 to 21 cm of soil surface due to soil-borne diseases, especially during heavy rainfall in summer, and that persisting plants were usually characterised by having a branched root system in upper soil layers.

In the studies reported herein, comparison of rooting characteristics of 3- and 6-weekly cut African and Florida 66 alfalfa were made by collecting root samples every 6 weeks and separating them into branch- and tap-roots. The ratio of branch-roots to total roots using their dry weights is shown in Tables 5 and 6 for the 1972 and 1973 seasons, respectively.

In 1972, mean ratios within cultivars at the two stages of maturity were not significantly different at $P < .05$. However, between cultivars, African had a significantly larger proportion of branch-roots than Florida 66 both in 1972 and 1973. Conversely, since total roots were made up of branch- and tap-roots, formed 81.5% and 85% of total roots in African and Florida 66 in 1972, respectively. In 1973 these proportions were 79% and 85%, respectively. In both years there were no heavy rains and therefore the incidence of soil-borne diseases on tap-roots was minimal. Thus, a stronger and more efficient tap-root system probably did contribute to the better performance of Florida 66 over African obtained in this experiment.

Number of Stems per Plant and Leaf to Stem Proportions

The number of stems (or shoots longer than 8 cm) per plant, and the leaf-to-stem ratio are presented in Tables 5 and 6. They were obtained from the same plants used in the determination of branch-root/tap-root proportions reported above. In 1972 there was no significant difference between the mean number of stems per plant within cultivars at both stages of maturity. However, between cultivars, African produced a significantly greater number of stems than Florida 66. Likewise, in 1973, African had a significantly higher number of stems than Florida 66 at the 6-week stage of maturity.

In 1972, 3-week old alfalfa plants had a higher leaf-to-stem ratio than 6-week old plants within cultivars. Between cultivars, African had a significantly higher leaf-to-stem ratio than Florida 66 at corresponding stages of maturity. However, in 1973, mean leaf-to-stem ratios of 6-week African and Florida 66 were similar.

Persistence of Stands

The average number of plants per meter or row at the beginning and end of each growing season is shown in Table 7. In 1972 Florida 66 had a significantly greater number of surviving plants than African at each corresponding stage of maturity. Likewise, in 1973, at the 6-week stage of maturity, Florida 66 had greater persistence than African. It is also evident that cutting at 3 weeks affected plant survival more adversely in African than Florida 66.

TABLE 1. SEASONAL DRY MATTER YIELDS (MT/HA) OF AFRICAN AND FLORIDA 66 ALFALFA AT TWO STAGES OF GROWTH IN 1972 AND 1973

Variety	Stage of Growth	Cuts *										Total
1972		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
African	3 wks	1.53	1.11	.82	.20	.11	.10	.10	.02	0	.05	4.04d**
African	6 wks	2.38	1.67	1.86	1.09	.70	-	-	-	-	-	7.70b
Florida 66	3 wks	1.60	1.44	.81	.20	.17	.20	.20	.08	0	.11	4.71c
Florida 66	6 wks	2.60	1.98	2.22	1.30	.80	-	-	-	-	-	8.90a
1973												
African	6 wks	1.62	2.08	1.34	.97	.22	-	-	-	-	-	6.23m
Florida 66	6 wks	2.22	2.38	1.64	1.42	.52	-	-	-	-	-	8.18n

* Average of 6 replications. Staging and frost-kill cuts not included

** Comparative values within each vertical column followed by the same letter are not significantly different at $P < .05$; $n > m$ at $t_{.05}$

TABLE 2. WEEKLY DRY WEIGHT OF REGROWTH AND PERCENT TNC IN THE ROOTS OF AFRICAN AND FLORIDA 66 ALFALFA AT TWO STAGES OF MATURITY IN THE 1972 GROWING SEASON

Maturity (weeks)	Weight (DM) of regrowth (mg)*	Date of sampling										
		4/20	4/27	5/4	5/9	5/16	5/23	5/30	6/6	6/13	6/20	6/27
3	African	1514	893	632	785	375	254	435	259	178	334	153
6	African	1639	1068	595	729	1192	1382	1299	792	363	663	818
3	Florida 66	1750	1043	580	636	494	205	338	233	189	355	239
6	Florida 66	1806	1183	711	676	917	1578	1578	884	516	790	895
	% TNC in roots**											
3	African	35.1	33.8	27.3	29.6	20.1	18.2	24.8	18.8	18.4	23.7	18.0
6	African	36.3	34.6	27.1	28.2	35.5	36.9	35.7	29.1	24.8	29.1	33.3
3	Florida 66	38.5	34.1	29.4	28.6	20.6	23.8	21.9	16.7	17.6	25.4	18.3
6	Florida 66	37.2	32.5	29.8	30.1	33.9	38.4	37.6	32.8	29.1	30.8	32.2

* Average of six plots (replications) per week
** Samples from six plots (replications) composited each week

TABLE 2 - CONTINUED

Maturity (weeks)	Weight (DM) of regrowth (mg)	Date of sampling 7/4	7/11	7/18	7/25	8/1	8/8	8/15	8/22	8/29	9/5	9/12
3	African	137	223	226	207	245	184	136	384	190	149	272
6	African	613	912	868	700	761	848	900	796	788	392	518
3	Florida 66	162	238	199	215	223	241	287	345	223	172	345
6	Florida 66	913	1008	894	726	732	763	1045	970	1071	622	763
	%TNC in roots											
3	African	17.4	19.7	13.0	13.1	18.4	12.2	17.4	23.7	16.0	14.6	19.7
6	African	34.2	30.8	24.4	25.8	27.4	34.9	36.7	36.6	32.1	25.3	27.9
3	Florida 66	16.9	20.0	14.8	16.0	22.9	21.3	19.6	28.2	23.6	19.0	16.0
6	Florida 66	34.8	34.3	30.4	29.4	31.7	36.8	38.1	42.0	38.4	29.4	32.0

TABLE 2 - CONTINUED

Maturity (weeks)	Weight (DM) of regrowth (mg)	Date of sampling									Mean***
		9/19	9/26	10/3	10/10	10/17	10/24	10/31	11/8	11/4	
3	African	260	110	152	177	221	495	412	333	460	9.3b
6	African	918	958	1299	702	524	852	1404	1432	1711	26.8a
3	Florida 66	272	171	305	210	315	410	301	280	478	9.7b
6	Florida 66	1035	1346	1367	787	596	1056	1259	1460	1834	29.9a
	%TNC in roots										
3	African	22.1	15.5	19.4	14.3	17.1	24.9	20.0	23.9	24.0	20.2c
6	African	31.0	31.9	35.8	29.9	29.2	32.7	34.4	35.2	34.9	31.6b
3	Florida 66	22.0	16.7	24.0	18.7	20.0	27.8	20.8	21.4	25.0	22.1c
6	Florida 66	37.1	34.3	40.3	36.9	33.8	34.7	36.6	36.8	38.7	34.5a

*** Mean of 30-week totals (g) for each of the six plots (reps.)
Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 3. PERCENT TOTAL NON-STRUCTURAL CARBOHYDRATES (TNC) IN THE ROOTS OF AFRICAN AND FLORIDA 66 ALFALFA CUT AND SAMPLED EVERY SIX WEEKS FROM 11/15/1972 TO 10/29/1973

	Topgrowth cutting and sampling date									
% TNC in	12/27	2/6	3/20	4/12	5/14	6/25	8/6	9/17	10/29	Mean
African	28.6	24.9	28.7	29.6	29.6	26.2	31.2	29.7	31.8	28.9m*
Florida 66	32.0	27.9	30.7	33.5	32.5	31.3	31.9	32.8	33.0	31.5n

* $n > m$ at $t_{.01}$

TABLE 4. REGRESSION EQUATIONS FOR THE PREDICTION OF %TNC (Y) USING DRY WEIGHT (IN GRAMS) OF DARK CHAMBER REGROWTH (X) IN AFRICAN AND FLORIDA 66 ALFALFA CUT AT TWO STAGES OF GROWTH

	Maturity (weeks)	Equation	$S_{y.x}$	r	F
African	3	$Y = 14.44 + 1.73X$	2.80	.87	93.2***
African	6	$Y = 24.22 + .81X$	2.82	.71	30.3***
Florida 66	3	$Y = 16.94 + 1.44X$	3.17	.82	59.8***
Florida 66	6	$Y = 27.76 + .66X$	2.72	.65	21.9***
African + Florida 66	3	$Y = 15.69 + 1.58X$	3.06	.84	142.6***
African + Florida 66	6	$Y = 25.50 + .78X$	2.92	.69	54.0***

*** Significant at $P < .001$
$S_{y.x}$ = Standard error of estimate
r = Correlation coefficient
F = Variance ratio

TABLE 5. EFFECT OF STAGE OF MATURITY ON LEAF, STEM AND ROOT PROPORTIONS IN AFRICAN AND FLORIDA 66 ALFALFA IN THE 1972 GROWING SEASON

Cultivar	Maturity (weeks)	Stem/plant	Dry weight proportions: leaf/stem	Dry weight proportions: branch roots/tap roots
African	3	3.8a*	.60a	.20a
African	6	4.1a	.52c	.19a
Florida 66	3	2.9b	.57b	.15b
Florida 66	6	2.9b	.48d	.15b

* Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 6. LEAF, STEM AND ROOT PROPORTIONS OF AFRICAN AND FLORIDA 66 ALFALFA CUT AT 6 WEEKS IN THE 1973 SEASON

	Cutting date					
	May 14	June 25	August 6	Sept. 17	Oct. 29	Mean
Shoots per plant						
African	7.4	4.9	4.7	5.8	6.2	5.8a
Florida 66	4.6	3.9	3.5	3.5	4.3	4.0b
Leaf/Stem ratio						
African	.51	.30	.30	.26	.31	.34
Florida 66	.47	.34	.31	.30	.30	.34
Branch roots/total roots						
African	.23	.22	.19	.24	.19	.21m
Florida 66	.14	.15	.15	.16	.15	.15n

a > b and m > n at $t_{.01}$

TABLE 7. EFFECT OF MATURITY STAGE ON THE PERSISTENCE OF AFRICAN AND FLORIDA 66 ALFALFA IN THE 1972 AND 1973 GROWING SEASONS

		Living plants/1 m row in 1972*			Living plants/1 m row in 1973*		
Cultivar	Maturity (wks)	April	November	% survival**	April	November	% survival
African	3	46	9	26.7d	-	-	-
African	6	53	25	43.6b	19	9	28.2b
Florida 66	3	59	24	39.4c	-	-	-
Florida 66	6	61	35	49.5a	28	15	33.6a

* Average of 6 replications

** Adjusted by arcsin transformation. Values within each vertical column followed by the same letter are not significantly different at $P < .05$ (November 1972) and at $t_{.05}$ (November 1973)

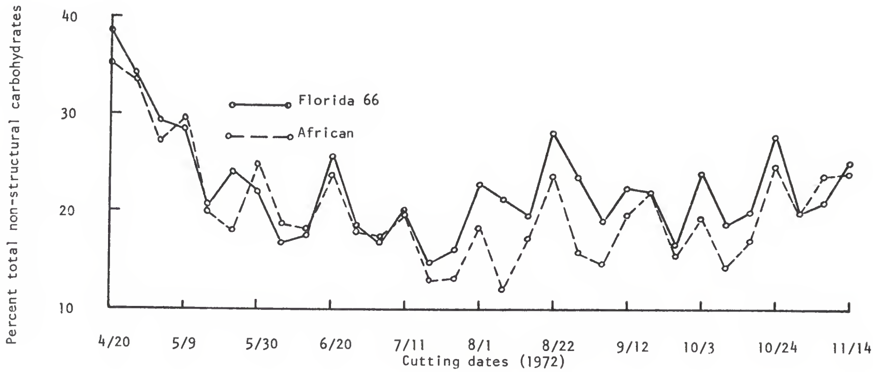


FIGURE 1. WEEKLY PERCENT TOTAL NON-STRUCTURAL CARBOHYDRATES OF AFRICAN AND FLORIDA 66 ALFALFA CUT AT 3 WEEKS OF MATURITY

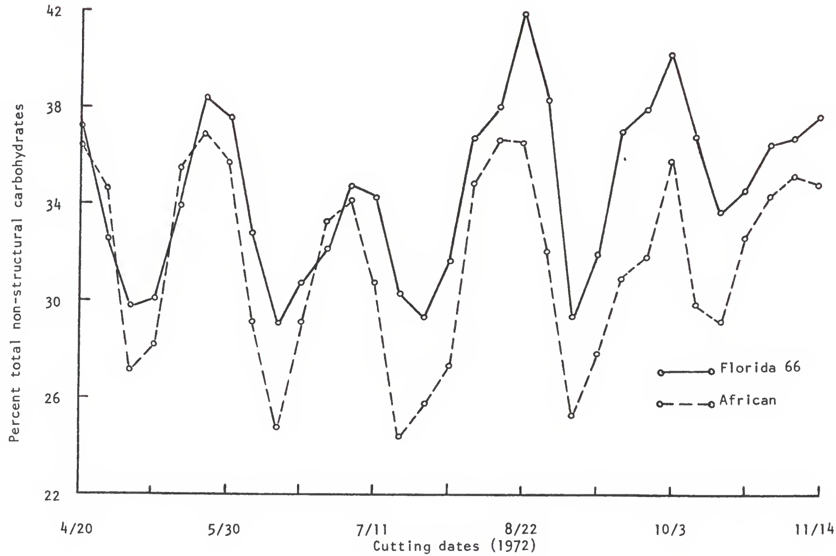


FIGURE 2. WEEKLY PERCENT TOTAL NON-STRUCTURAL CARBOHYDRATES OF AFRICAN AND FLORIDA 66 ALFALFA CUT AT 6 WEEKS OF MATURITY

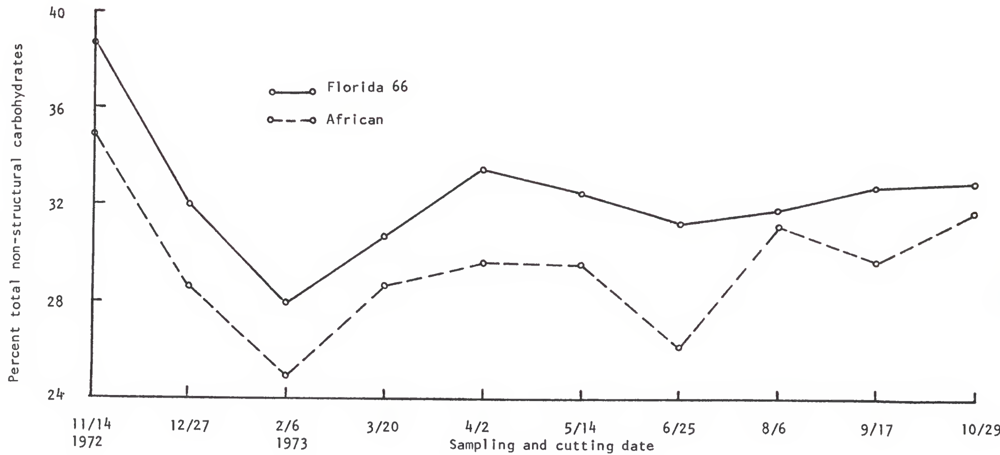


FIGURE 3. PERCENT TNC IN THE ROOTS OF AFRICAN AND FLORIDA 66 ALFALFA CUT AT 6 WEEKS OF MATURITY FROM FALL 1972 TO FALL OF 1973

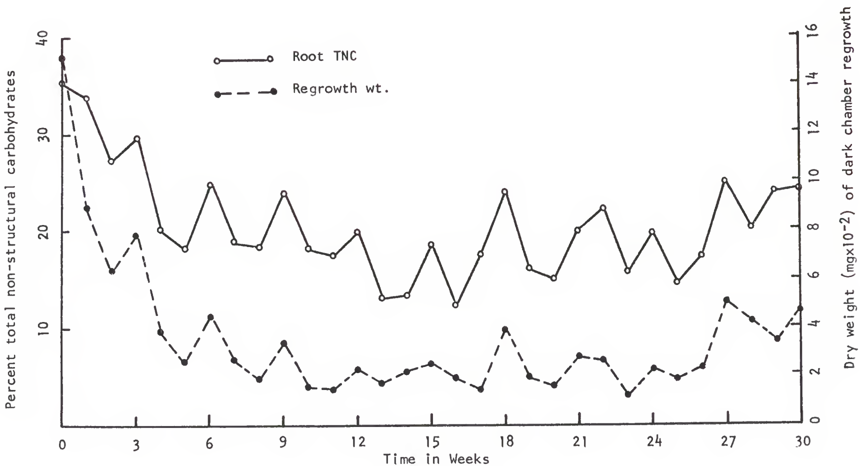


FIGURE 4. WEEKLY PERCENT TOTAL NON-STRUCTURAL CARBOHYDRATES AND DRY WEIGHT OF DARK CHAMBER REGROWTH OF AFRICAN ALFALFA CUT AT 3 WEEKS OF MATURITY

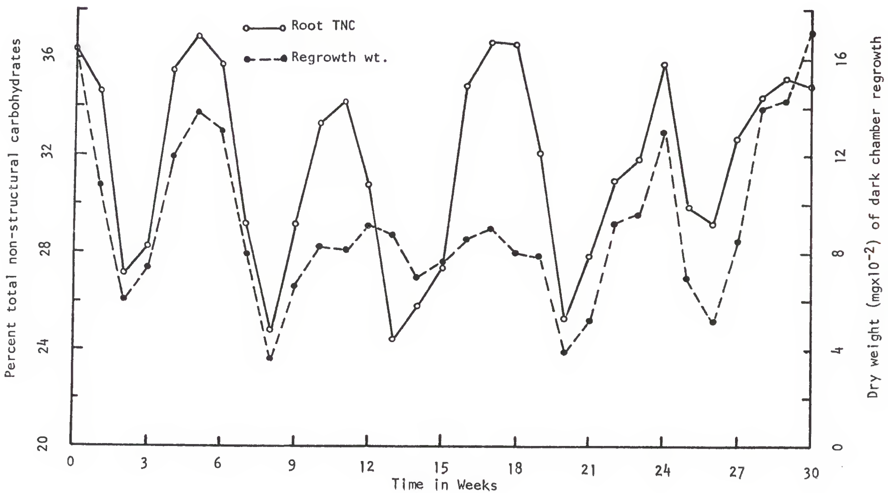


FIGURE 5. WEEKLY PERCENT TOTAL NON-STRUCTURAL CARBOHYDRATES AND DRY WEIGHT OF DARK CHAMBER REGROWTH OF AFRICAN ALFALFA CUT AT 6 WEEKS OF MATURITY

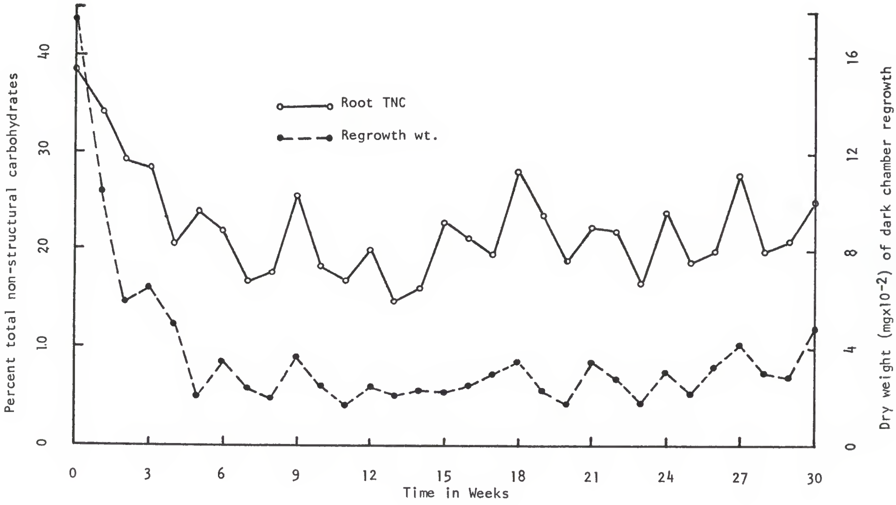


FIGURE 6. WEEKLY PERCENT TOTAL NON-STRUCTURAL CARBOHYDRATES AND DRY WEIGHT OF DARK CHAMBER REGROWTH OF FLORIDA 66 ALFALFA CUT AT 3 WEEKS OF MATURITY

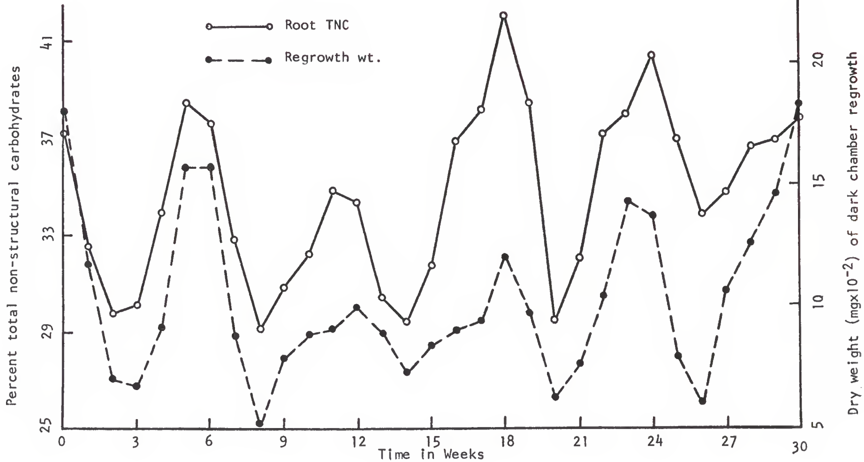


FIGURE 7. WEEKLY PERCENT TOTAL NON-STRUCTURAL CARBOHYDRATES AND DRY WEIGHT OF DARK CHAMBER REGROWTH OF FLORIDA 66 ALFALFA CUT AT 6 WEEKS OF MATURITY

FIGURE 8. REGROWTH OF AFRICAN AND FLORIDA 66 ALFALFA IN THE DARK CHAMBER

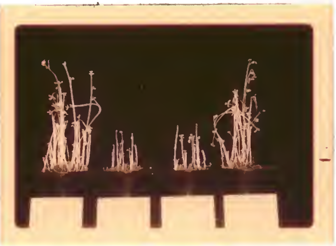
A - 1st day in May, 1972

B - 12th day in May, 1972
Similar regrowths at the two cutting intervals

C - 12th day in October, 1972
(Left to right): Florida 66 cut at 6 and 3 weeks, and African cut at 3 and 6 weeks

A

B



C

CHAPTER IV

EXPERIMENT II

THE EFFECT OF RATE AND FREQUENCY OF FERTILIZER APPLICATION ON HERBAGE YIELD, PERSISTENCE, AND CHEMICAL COMPOSITION OF FLORIDA 66 ALFALFA

Materials and Methods

Florida 66 alfalfa was seeded on October 12, 1971, at the same rate, row spacing and on a similarly treated seedbed as described in Experiment I. Just before seeding, 48.8 plus 186 kg/ha of P and K, respectively, 22.4 kg/ha of borax and 11.2 kg/ha of FTE 503 (containing approximately .34, 2.2, .82 and .78 kg of Cu, Fe, Mn and Zn, respectively) were disked into the top soil of the seedbed. The 0-10-20 ($N-P_2O_5-K_2O$) fertilizer, mixed with FTE 503, was used (analysis shown in Table A-8) to make up the following treatments for each of the 1972 and 1973 seasons:

Treatment	No. of Applications	Kg/ha/year P	Kg/ha/year K
1	-	0	0
2	1 in spring	48.8	186
3	1 in spring	97.6	372
4	4 equal splits	24.4	93
5	4 equal splits	48.8	186
6	4 equal splits	97.6	372

The amounts of micronutrients from FTE 503 which was mixed in the fertilizer were approximately as follows (Kg/ha):

Treatment	B	Cu	Fe	Mn	Zn	Mo
1	0	0	0	0	0	0
2	4.86	.34	2.16	.82	.78	.02
3	7.29	.51	3.24	1.23	1.07	.03
4	2.43	.71	1.08	.41	.39	.01
5	4.86	.34	2.16	.82	.78	.02
6	7.29	.51	3.24	1.23	1.07	.03

Spring applications were made shortly after the staging cut in April. Split applications were made after the staging, 2nd, 4th and 6th cutting. Treatments were randomly distributed within blocks, with six replications. All treatments were cut at first flower. Forage dry matter yields and persistence of stands were determined as in Experiment I. Dried forage samples were ground to pass through a 1-mm screen in a Wiley mill and stored in sealed polyethylene bags until analysis. Analysis for N was carried out using the Technicon Autoanalyzer II for Kjeldahl N determination (213). Phosphorus, K, Ca and Mg and the micronutrients (Fe, Cu, Zn, Mn) were determined at the Soils Laboratory, University of Florida, using A.O.A.C. procedures (9). Similar procedures were used in determining the levels of P, K, Ca and Mg in soil samples obtained from the 0 to 15 cm layer of each treatment plot at the beginning and end of each season.

The analysis of variance of a randomized complete block design was used according to LeClerg et al. (124) and mean separation according to Duncan's new multiple range test (69).

Results and Discussion

Results of herbage dry matter yields, persistence of stands and percent and total amounts of N, P, K, Ca, Mg, Fe, Cu, Zn and Mn in harvested Florida 66 alfalfa were obtained over a period of two years.

As described in Experiment I, the first growth of Florida 66 in Experiment II was also cut on January 19, 1972 to remove frost-damaged herbage. The next cutting was made at staging on April 18, 1972 when the plants were in mid-bloom. Herbage removed averaged 2.8 MT/ha of dry matter.

The last cutting in the first season was made on November 27, 1972. Subsequent regrowth was cut on January 16, 1973 after the frost damage of January 14, 1973. The next cutting was made at the second staging on April 2, 1973, which averaged 1.3 MT/ha of dry matter for all the treatments.

Dry matter yields of frost-kill and staging cuts were not included in treatment response analyses. All significant and non-significant differences quoted herein are at the $P<.05$ level of significance.

Dry Matter Yields

The seasonal and total dry matter yields of Florida 66 at

different fertilizer rates and frequencies are presented in Tables 8 and 9 for the 1972 and 1973 growing seasons, respectively. In each season, a total of seven cuts were made from each of the treatments, which were cut at the first flower stage of development.

In the 1972 season total DM yields from treatments 2, 3, 5 and 6, in which fertilizer applications were made once and in four splits, were not significantly different. However, treatment 4 produced significantly less total DM yield than the four treatments above, while the zero fertilizer treatment had significantly the lowest total DM yield. All yields declined as the growing season progressed.

In the 1973 season, four split applications of 97.6 and 372 kg/ha of P and K, respectively, produced 7.6 MT/ha of dry matter which was significantly the highest. The zero fertilizer treatment produced 2.3 MT/ha of dry matter which, as in the 1972 season, was significantly the lowest yield.

For both seasons, the highest total dry matter yields of 15.4 and 14.3 MT/ha were from the highest fertilizer rates (treatments 6 and 3, respectively), while 8.53 MT/ha from the zero fertilizer treatment was significantly the lowest.

These data show that Florida 66 alfalfa positively responded to fertilizer application as dry matter yields increased with increasing levels of fertilization up to the highest rate of 97.6 and 372 kg/ha of P and K, respec-

tively. Secondly, differences in response between treatments were more pronounced in the second, than in the first, harvest season. In the first harvest season there was no significant difference in response between and within the two highest rates applied in single or split applications. However, in the second season, split applications of these gave higher yields than their respective single application treatments in the first season.

Ruelke and Prine (180) reported similar findings in Florida, i.e., lack of significant difference in yields of Florida 66 alfalfa between fertilizer rates in the first harvest season, and more response from split fertilizer applications, especially in the third season.

Persistence of Stands

The average number of plants per meter of row at the beginning and end of each season (1972 and 1973) is shown in Table 10. The number of surviving plants at the end of each season was expressed as percentages of the original stand counts and tranformed to arcsin values before the analysis of variance (135).

At the two highest fertilizer rates, plant survival was greater from single than from split applications at corresponding rates in 1972. In 1973, the highest fertilizer rate in single application again had the highest plant survival rate. In 1972 plant survival was lowest in treatment 4 (split applications), followed by the zero fertilizer treat-

ment. By the end of 1973, the zero fertilizer treatment was the lowest

In general, plant persistence was more enhanced by higher fertilizer rates in the first and second seasons. The relationship between plant persistence (Table 10) and herbage dry matter yields (Tables 8 and 9) seems to be more positively associated with increasing fertilizer rates than with split vs single applications. Previously, Ruelke and Prine (180) had reported from a 3-year study that the greatest reduction in number of plants occurred where all the fertilizer was applied in one application in the fall, indicating the desirability of split applications. The number of surviving plants also tended to increase with increasing rates of fertilization but, generally, seasonal differences between fertilizer rates were not significant.

Mineral Composition

The composition of nine mineral elements in Florida 66 alfalfa hay at different rates and frequencies of fertilization is presented in Tables 11 and 12 for the 1972 and 1973 seasons, respectively.

Ca and Mg

The main source of Ca and Mg was dolomitic limestone which was applied at the rate of 1120 kg/ha on September 13, 1971, one month before seeding. The main purpose of liming was to correct soil acidity to a pH range of between 6.5 and

7.5 reported (38, 255) as being ideal for maximum alfalfa production. Lime was also a source of plant nutrients, Ca and Mg. Table A-1 shows that average pH values of soils from all treatments at the beginning and end of the 1972 season were between 6.6 and 6.8. In 1973 soils from all treatments had mean pH values of 6.6 and 6.3 at the beginning and end of the season, respectively.

In Tables 11 and 12, percentages of Ca and Mg in plant tissue decreased with increasing amount of fertilizer both in 1972 and 1973, the 1973 levels being higher than at corresponding treatments in 1972. These concentrations are within the range, 1 to 1.5% for Ca and .30% for Mg, reported as normal composition in the dry matter of alfalfa herbage (133, 173).

Amounts of Ca and Mg removed in harvested alfalfa are shown in Tables 13 and 14. These increased with increasing amounts of fertilizer. The amount of Ca and Mg in the top 15 cm of soil shown in Tables A-2 and A-3 indicate that there was very little change in composition in the two years, the amounts found in plant tissue being only a small fraction.

<u>Fe, Zn, Cu, and Mn</u>

The main source of these four micronutrients was fritted trace elements (FTE 503) applied just before seeding in October 1971, and thereafter, FTE 503 was applied in mixture with the 0-10-20 fertilizer, at the rates shown in the Materials and Methods section.

Adequate levels of Fe, Zn, Cu, and Mn in alfalfa have been listed as 200, 21 to 70, 5 to 14, and 20 to 100 ppm, respectively (133, 173). Tables 11 and 12 show that the levels of these micronutrients were within the adequate range. The amounts of Fe, Zn, Cu and Mn removed in harvested herbage also increased in proportion to dry matter yields, which in turn increased with increasing levels of fertilization.

N, P and K

Average composition of N in alfalfa has been reported as 3% (152) and adequate levels of P and K as .20 to .40% and +2.0%, respectively (133). Tables 11 and 12, show that %N decreased slightly with increasing fertilizer rates in both seasons, but these differences were not statistically significant. Likewise, the concentration of P in 1972 and 1973 were similar at all levels of fertilization. On the other hand, the concentration of K in alfalfa significantly increased with increasing levels of fertilization. The zero fertilizer treatment in 1972 and 93 kg/ha of K in split applications in 1973 fell below the adequate level.

A similar relationship between N and K was reported by Dionne (63) and Klebesedale (116) who found that the percentage of crude protein (%N x 6.25) in alfalfa was unaffected or slightly depressed, while dry matter yield and total CP production increased with increasing rates of K fertilization. This is not unexpected since K fertilization increases DM production by producing taller and larger

alfalfa plants which have comparatively more stem to leaf ratio and, therefore, increased structural components and reduced CP concentration (134).

Tables A-4 and A-5 show the amounts of ammonium acetate (PH 4.8) extractable P (kg/ha) found in the surface 15 cm of soil at the start and end of the 1972 and 1973 seasons, respectively. Also shown are total amounts applied and in alfalfa herbage. In both years there was no significant response to different rates and frequencies of P fertilization. Concentrations of P in alfalfa herbage (Tables 11 and 12) were similar and above the adequate level of 0.30%. This indicated that the soils of the location where this study was conducted had adequate supplies of available P for alfalfa growth.

The amount of K (kg/ha) found in the surface 15 cm of soil at the start and end of the 1972 and 1973 growing seasons are shown in Tables A-6 and A-7, respectively. When the amounts of K in alfalfa herbage were compared with total amounts of K in the fertilizer source, it was apparent that the amounts of K that were taken up by alfalfa were nearly as high as, and sometimes more than, the amount of K in the fertilizer. The concentration of K in alfalfa and DM yield of alfalfa were significantly increased by increasing levels of fertilization up to the highest fertilizer rate (372 kg/ha). Therefore, after meeting the basic requirements for pH and micronutrients,

K was the most likely nutrient to be limiting in alfalfa production on this soil.

TABLE 8. DRY MATTER YIELD (MT/HA) OF FLORIDA 66 ALFALFA IN RESPONSE TO DIFFERENT FERTILIZER RATES AND FREQUENCIES DURING THE 1972 GROWING SEASON

Treat-ment	No. of Appli-cations	Kg/ha/yr		Cuts*							Total
		P	K	1st	2nd	3rd	4th	5th	6th	7th	
1	-	0	0	2.01	1.05	1.23	.57	.43	.41	.54	6.24c***
2	1 in spring	48.8	186	2.13	1.29	1.49	.78	.61	.55	.64	7.49a
3	1 in spring	97.6	372	2.13	1.52	1.58	.84	.61	.61	.65	7.94a
4	4 equal splits	24.4	93	2.03	1.09	1.35	.61	.53	.57	.68	6.86b
5	4 equal splits	48.8	186	2.00	1.25	1.50	.84	.63	.65	.72	7.60a
6	4 equal splits	97.6	372	2.08	1.12	1.64	.84	.67	.67	.77	7.78a

* Average of 6 replications. Staging and frost-kill cuts not included.

** Values followed by the same letter are not significantly different at $P < .05$

TABLE 9. DRY MATTER YIELD (MT/HA) OF FLORIDA 66 ALFALFA IN RESPONSE TO DIFFERENT FERTILIZER RATES AND FREQUENCIES DURING THE 1973 GROWING SEASON

Treat- ment	No. of Appli- cations	Kg/ha/yr		Cuts*							1973
		P	K	1st	2nd	3rd	4th	5th	6th	7th	Total
1	-	0	0	.82	.76	.30	.16	.14	.08	.03	2.29d**
2	1 in spring	48.8	186	1.44	1.20	1.08	.58	.43	.28	.19	5.32bc
3	1 in spring	97.6	372	1.63	1.30	1.26	.79	.62	.43	.32	6.38b
4	4 equal splits	24.4	93	1.36	1.00	.75	.46	.32	.25	.18	4.32c
5	4 equal splits	48.8	186	1.64	1.36	1.33	.66	.50	.42	.30	6.21b
6	4 equal splits	97.6	372	1.94	1.47	1.37	.98	.79	.58	.41	7.57a

* Average of 6 replications. Staging and frost-kill cuts not included.

** Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 10. EFFECT OF DIFFERENT RATES AND FREQUENCIES OF FERTILIZATION ON THE PERSISTENCE OF FLORIDA 66 IN THE 1972 AND 1973 GROWING SEASONS

Treat-ment	No. of Appli-cations	Kg/ha/yr		Plants/1 m row in 1972			Plants/1 m row in 1973		
		P	K	Apr.	Nov.	% survival	Apr.	Nov.	% survival
1	-	0	0	65	28	41.1c	21	9	22.1d
2	1 in spring	48.8	186	58	30	45.9b	26	14	26.9c
3	1 in spring	97.6	372	63	35	48.8a	21	16	32.0a
4	4 equal splits	24.4	93	68	27	39.6d	23	12	26.2c
5	4 equal splits	48.8	186	63	31	44.4b	20	13	27.5b
6	4 equal splits	97.6	372	62	30	44.7b	22	15	28.8b

* Percentages of surviving plants transformed to arcsin values

** Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 11. MINERAL CONTENT OF FLORIDA 66 ALFALFA AT DIFFERENT RATES AND FREQUENCIES OF FERTILIZATION IN 1972

Treat-ment	No. of Appli-cations	Kg/ha/yr		%	%	%	%	%	ppm	ppm	ppm	ppm
		P	K	N	P	K	Ca	Mg	Fe	Zn	Cu	Mn
1	-	0	0	3.37a	.34a	1.70d	1.10	.32	155	31	15	28
2	1 in spring	48.8	186	3.32a	.32a	2.49ab	1.02	.26	142	29	15	28
3	1 in spring	97.6	372	3.29a	.32a	2.56a	.97	.24	158	26	13	28
4	4 equal splits	24.4	93	3.43a	.34a	2.11c	1.08	.31	155	30	15	30
5	4 equal splits	48.8	186	3.40a	.34a	2.23bc	1.08	.30	164	30	15	29
6	4 equal splits	97.6	372	3.37a	.31a	2.53a	.97	.25	163	30	14	28

Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 12. MINERAL CONTENT OF FLORIDA 66 ALFALFA AT DIFFERENT RATES AND FREQUENCIES OF FERTILIZATION IN 1973

Treat-ment	No. of Appli-cations	Kg/ha/yr P	Kg/ha/yr K	% N	% P	% K	% Ca	% Mg	ppm Fe	ppm Zn	ppm Cu	ppm Mn
1	-	0	0	3.90a*	.35a	1.67d	1.27	.36	152	49	18	30
2	1 in spring	48.8	186	3.75a	.36a	2.59ab	1.21	.31	133	45	16	31
3	1 in spring	97.6	372	3.79a	.35a	2.85a	1.12	.30	132	44	14	34
4	4 equal splits	24.4	93	3.89a	.35a	1.98c	1.18	.34	139	40	15	29
5	4 equal splits	48.8	186	3.85a	.36a	2.41b	1.20	.31	134	49	14	28
6	4 equal splits	97.6	372	3.82a	.35a	2.85a	1.11	.28	123	58	16	33

* Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 13. QUANTITIES OF MINERAL ELEMENTS REMOVED IN FLORIDA 66 ALFALFA HERBAGE AT DIFFERENT FERTILIZER RATES AND FREQUENCIES IN THE 1972 SEASON

Treatment	No. of Applications	Kg/ha/yr		Kg/ha/yr								
		P	K	N	P	K	Ca	Mg	Fe	Zn	Cu	Mn
1	-	0	0	235c*	23.9c	119d	76.9	22.4	1.1	.22	.11	.20
2	1 in spring	48.8	186	278ab	26.8ab	209ab	85.6	21.8	1.2	.24	.13	.24
3	1 in spring	97.6	372	292a	28.2a	227a	86.2	21.3	1.4	.23	.12	.25
4	4 equal splits	24.4	93	263b	26.1b	165c	82.9	23.8	1.2	.23	.12	.23
5	4 equal splits	48.8	186	290a	28.5a	191bc	92.0	25.5	1.4	.26	.13	.25
6	4 equal splits	97.6	372	293a	27.2ab	222ab	84.0	21.8	1.4	.26	.12	.24

* Values in each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 14. QUANTITIES OF MINERAL ELEMENTS REMOVED IN FLORIDA 66 ALFALFA HERBAGE AT DIFFERENT FERTILIZER RATES AND FREQUENCIES IN THE 1973 GROWING SEASON

Treat- ment	No. of Appli- cations	Kg/ha/yr		Kg/ha/yr								
		P	K	N	P	K	Ca	Mg	Fe	Zn	Cu	Mn
1	-	0	0	100d*	9.3d	45e	32.6	9.2	.39	.13	.046	.076
2	1 in spring	48.8	186	224bc	21.6b	156c	72.1	18.5	.79	.27	.095	.185
3	1 in spring	97.6	372	271ab	25.3b	206ab	80.0	21.4	.94	.31	.100	.243
4	4 equal splits	24.4	93	188c	16.9c	95d	57.2	16.5	.67	.19	.073	.141
5	4 equal splits	48.8	186	268b	25.0b	171bc	83.5	21.6	.93	.34	.097	.195
6	4 equal splits	97.6	372	324a	29.7a	244a	94.2	23.7	1.04	.49	.136	.280

* Values in each vertical column followed by the same letter are not significantly different at $P < .05$

CHAPTER V

EXPERIMENT III

YIELDS, STAND PERSISTENCE AND QUALITY OF FLORIDA 66 ALFALFA SUBJECTED TO SIX DIFFERENT HARVESTING SCHEDULES

Materials and Methods

Florida 66 alfalfa was established as in Experiment I. All plots received four fertilizer applications, each of 48.8 and 186 kg/ha of P and K, respectively, and 5.6 kg/ha of FTE 503 at six weeks intervals during each growing season.

Three harvesting criteria, namely, calendar date, stage of growth and accumulated heat units were compared at two levels of each, making a total of the following six different harvesting schedules:

Treat-ment	Harvesting criterion	Harvest schedule
1	Calendar date	3 weeks
2	Calendar date	6 weeks
3	Stage of growth	1st bloom
4	Stage of growth	Full bloom
5	Heat units	1000 h.u.
6	Heat units	1500 h.u.

Heat units (h.u.) were computed by substracting the base temperature of 40F (4.4C) from the mean of maximum and minimum temperature of each day and adding these up until totals of 1000 or 1500 h.u. were accumulated. Weather data were collected from a meteorological station (Gainesville 2 WSW) situated about 100 meters from the plots. A summary of monthly temperature and rainfall for the two years is shown in Table A-9.

The six treatments were randomly distributed into each of the six blocks (replications). Herbage DM yields, persistence of stands and crude protein percentage were determined as in Experiment II. Percent _in vitro_ organic matter digestion (IVOMD) was determined at the Forages Laboratory, University of Florida, using the two-stage technique of Tilley and Terry which was modified by Moore et al. (144).

Analysis of variance of a randomized block design was carried out according to LeClerg et al. (124) and treatment mean separation by Duncan's new multiple range test (69).

Results and Discussion

The effects of cutting Florida 66 alfalfa at two intervals within three different criteria, namely, fixed calendar date, physiological stage of maturity and accumulated heat units, were determined in terms of dry matter yields, persistence of stands, CP and IVOMD over a period of two years.

As described in Experiment I, the first growth of Florida 66 in Experiment III was also cut on January 19, 1972, to

remove frost-damaged herbage. The next cutting was made at staging on April 18, 1972 when plants were in mid-bloom. Herbage removed averaged 2.6 MT/ha of dry matter.

The last cutting in the first season was made between November 2 and 27, 1972. Subsequent regrowth was cut on January 16, after the frost damage of January 14, 1973. The next cutting was made at the second staging on April 2, 1973, which averaged 1.3 MT/ha of dry matter for all the treatments.

Dry matter yields of frost-damaged herbage and staging cuts mentioned above were not included in treatment response analyses. All significant and non-significant differences mentioned herein are at the $P<.05$ level of significance.

Number of Days and Total Heat Units Between Cutting Intervals

Tables 15 and 16 show the length of intervals between different cutting treatments of Florida 66 alfalfa in the 1972 and 1973 seasons, respectively. The 3- and 6-week schedules were fixed at 21 and 42 days. In both seasons, the first flower stage varied from 26 to 35, with a mean of 28 days. The full bloom stage varied from 27 to 42, with a mean of 36 days. The 1000 h.u. schedule varied from 21 to 38, with a mean of 27 days, and the 1500 h.u. schedule from 33 to 56, with a mean of 40 days. In terms of weeks, the first flower and 1000 h.u. schedules averaged 4 weeks, while the full bloom and 1500 h.u. averaged 5 and 6 weeks, respectively.

From a practical and management point of view, fixed cutting schedules, i.e., 3- and 6-week intervals, were the easiest to follow. By just marking them off the calendar, it was possible to plan ahead and to fit in other farm operations. It was not necessary to visit the field as often as demanded when using the flowering criterion to determine when to cut the alfalfa. Thus, fixed cutting schedules ran the risk of mismanagement through infrequent visits, especially where it was necessary to check the crop for weeds, pests and diseases, or to determine irrigation needs.

Cutting intervals based on accumulated heat units were generally longer during the cooler parts of the season, i.e., mid-spring and the first half of fall. Like the fixed cutting date schedule, the h.u. system did not require visiting the crop at all. Cutting dates could not be precisely predicted. However, this system kept one more attuned to weather trends, and required diligent attention in computing h.u. from daily temperatures.

Tables 17 and 18 present total h.u. accumulated during intervals between different cutting schedules of Florida 66 alfalfa in 1972 and 1973, respectively. The 1000 and 1500 h.u. treatments were fixed. In both years, the 3- week stage of maturity ranged from 490 and 892, with a mean of 780, h.u. The 6- week stage ranged from 1165 to 1770, with a mean of 1571, h.u. The first flower stage of maturity ranged from

784 to 1399, with a mean of 1112 h.u., while the full bloom stage ranged from 1026 to 1740, with a mean of 1385 h.u. At the 3- and 6-week fixed intervals, fewer h.u. were accumulated between cuts during mid-spring and the first half of fall, i.e., the cooler parts of the growing season. With flowering stages of development, especially the first flower, total h.u. fluctuated around their respective seasonal means.

Like the h.u. systems, cutting intervals between flowering stages were generally longer at the beginning and toward the end of the growing seasons. The first flower and full bloom stages were fairly easy to recognize although, often, with some degree of subjectivity. However, the system made one more acquainted with the development of the crop since frequent visits were necessary in order to observe and determine whether and when plants were at first flower or in full bloom.

Dry Matter Yields

Dry matter (DM) yields of Florida 66 alfalfa at different cutting criteria are presented in Tables 19 and 20 for the 1972 and 1973 growing seasons, respectively. The number of cuts per treatment ranged from 5 to 10 in 1972 and from 5 to 9 in 1973. In both years, DM production decreased from the beginning to the end of the growing season. Secondly, total DM production for each treatment was higher in 1972 than in 1973.

In 1972 alfalfa cut at 6 weeks of maturity had a total DM yield of 9.2 MT/ha which was the highest. The 1500 and 1000 h.u. treatments produced the second and third highest yields. The full bloom treatment produced the next DM yield which was similar to those of the two heat unit treatments. The second lowest yield was obtained from alfalfa cut at the first flower stage of development. The 3-week cutting interval produced a total of 5.2 MT/ha, which was significantly the lowest yield. In 1973, the 6-week cutting interval produced 8.8 MT/ha of total DM which was, again, significantly the highest yield. The 3-week cutting interval produced 2.2 MT/ha which was also significantly the lowest. Cutting at full bloom and at 1500 h.u. produced the second highest yield, while DM yields from 1000 h.u. and first flower treatments were the second lowest.

Clearly, therefore, dry matter yields of Florida 66 alfalfa were greatly influenced by the three cutting criteria. The development of alfalfa underfixed cutting intervals and cutting intervals under fixed heat units and flowering stages were all influenced by changes in weather, especially temperature. Similar trends in dry matter production have been reported by Jones (105), Weir et al. (240), and Peterson and Hagan (164). Their largest total DM yields were obtained when alfalfa was cut at a 5-week interval in comparison with either 2-, 3-, or 4-week cutting interval. With respect to the flowering stage, dry matter yields increased per cutting up until the time of full bloom.

Percentages and Yield of CP

The percentage of CP in Florida 66 alfalfa at different cutting schedules is shown in Table 21. In 1972, 3-week alfalfa had 24.0% which was significantly the highest. However, total CP which was 1.2 MT/ha in harvested alfalfa was significantly the lowest. Treatments which had longer cutting intervals, namely, 6-week, full bloom and 1500 h.u., had lower CP percentages (approximately 18.0%), but higher CP yield, than treatments cut at shorter intervals. In 1973, 3-week alfalfa again had the highest CP and the lowest yield of CP per hectare. First flower and full bloom treatments had similar CP but full bloom had a significantly higher CP yield which, together with that of the 6-week alfalfa, were significantly the highest. As in 1972, treatments with longer cutting intervals had lower CP, the 1500 h.u. treatment had 22.7% which was the lowest. In all cases, CP in 1973 was several units higher than at corresponding treatments in 1972. This might have been due to a more developed root/rhizobia system concentrating their N into plants whose sizes were visibly a little less than those of the first season.

Average length of intervals (in days) between cuts of the six treatments has been shown in Tables 15 and 16. Data in Table 21 clearly indicate that CP in Florida 66 alfalfa decreased with advancing maturity, or as the cutting interval was lengthened. The yield of CP per hectare was influenced

more by DM yield per hectare than by the percentage of CP in herbage. The lowest concentration of CP was 17.9% in 1972, and 23.9% in 1973, both from the 6-week alfalfa. This demonstrates the value of alfalfa as a source of protein which, even at an advanced stage of maturity, is still considerably higher than can be found in most other feeds at comparative or lower stages of maturity (52).

Percent IVOMD and Yield of DOM

Data on percent IVOMD and digestible organic matter (DOM) per hectare in Florida 66 at different cutting treatments are presented in Table 22. With reference to Tables 15 and 16 (cutting intervals) and Table 21(percentage and yield of CP), the percentage of IVOMD, like that of CP, decreased with advancing maturity, or as the cutting interval was lengthened. Secondly, percentages of IVOMD in 1973, like CP, were higher than at corresponding treatments in 1972. Similarly, in both years, the yield of DOM was partly influenced by %IVOMD, but largely by total DM yield per hectare. For the two-year period, 3-week alfalfa averaged 73.8% IVOMD, which was the highest, but its DOM yield was significantly the lowest. In 1973 6-week alfalfa averaged 67.2% IVOMD which was the lowest. However, 6-week alfalfa had the highest DOM in 1972 and 1973. This further illustrates the value of Florida 66 alfalfa as a feed with high concentration and yield of protein, digestibility and DOM, even at advanced stages of maturity.

Persistence of Stands

The average number of plants per meter of row at the beginning and end of each growing season is shown in Table 23. The number of surviving plants at the end of each season was expressed as percentages of the original stand counts and transformed to arcsin values before the analysis of variance, according to LeClerg et al. (124). With reference to Tables 15 and 16 (intervals between cuts) it is clear that in both years the persistence of Florida 66 was generally better when the cutting intervals were longer. Alfalfa cut at 3 weeks of age had significantly the lowest, while cutting at full bloom had the highest, survival rate. The 6-week alfalfa had the second highest survival rate both in the first and second seasons. The survival rate of alfalfa in the 1500 h.u. treatment was only significantly higher than that of the 1000 h.u. treatment in the second season.

The goal of the alfalfa producer is to obtain the largest yield of high quality forage consistent with reasonable stand survival. Hence, in selecting the best cutting criterion, plant survival data should be evaluated in conjunction with other components of yield. In Tables 19, 20, 21 and 22 6-week alfalfa had the highest yield of DM, CP and DOM per hectare. On the other hand, yields of DM, CP and DOM from full bloom alfalfa whose cutting intervals averaged 5 weeks and which had the highest plant survival rate, were slightly less than those of the alfalfa cut at 6 weeks of maturity.

Some components of yield of the 1500 h.u. treatment were similar to, and others higher than, those of the full bloom alfalfa.

Smith et al. (203) have stressed the importance of cutting alfalfa based on its physiological stage of development, i.e., the flowering stage. However, in Florida, high day and night temperatures not only enhance rapid development to flowering but also loss of carbohydrate reserves through respiration. Therefore, on the basis of flowering stage, alfalfa would be cut at shorter, rather than longer, intervals which appear to be necessary for building up sufficient carbohydrate reserves before the next cutting. In light of the findings of this experiment, the 6-week cutting interval appears to have favored the highest productivity and reasonable persistence of Florida 66 alfalfa during the first two years of growth.

TABLE 15. LENGTH OF INTERVALS (IN DAYS) BETWEEN CUTS OF FLORIDA 66 ALFALFA UNDER DIFFERENT HARVESTING SCHEDULES IN THE 1972 GROWING SEASON

Harvesting schedule	Cuts										Mean/cut
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
3 weeks	21	21	21	21	21	21	21	21	21	21	21
6 weeks	42	42	42	42	42	-	-	-	-	-	42
1st flower	28	28	32	28	28	35	30	40	-	-	32
Full bloom	35	35	35	42	49	-	-	-	-	-	39
1000 h.u.*	30	28	24	24	24	25	28	37	-	-	28
1500 h.u.	45	37	36	37	42	-	-	-	-	-	39

* h.u. = heat units

TABLE 16. LENGTH OF INTERVALS (IN DAYS) BETWEEN CUTS OF FLORIDA 66 ALFALFA UNDER DIFFERENT HARVESTING SCHEDULES IN THE 1973 GROWING SEASON

Harvesting schedule	Cuts										Mean/cut
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
3 weeks	21	21	21	21	21	21	21	21	21	21	21
6 weeks	42	42	42	42	42	-	-	-	-	-	42
1st flower	30	26	26	29	28	31	31	-	-	-	29
Full bloom	41	27	34	30	35	38	-	-	-	-	34
1000 h.u.	38	23	27	21	22	24	28	-	-	-	26
1500 h.u.	56	33	34	34	48	-	-	-	-	-	41

TABLE 17. TOTAL HEAT UNITS ACCUMULATED DURING INTERVALS BETWEEN DIFFERENT HARVESTING SCHEDULES OF FLORIDA 66 ALFALFA DURING THE 1972 GROWING SEASON

Harvesting schedule	Cuts										Mean/cut
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
3 weeks	650	726	798	859	878	892	806	842	703	682	784
6 weeks	1376	1657	1700	1648	1385	-	-	-	-	-	1567
1st flower	904	988	1306	1181	1399	1107	1131	-	-	-	1145
Full bloom	1135	1319	1457	1740	1733	-	-	-	-	-	1477
1000 h.u.	1000	1000	1000	1000	1000	1000	1000	1000	-	-	1000
1500 h.u.	1500	1500	1500	1500	1500	-	-	-	-	-	1500

TABLE 18. TOTAL HEAT UNITS ACCUMULATED DURING INTERVALS BETWEEN DIFFERENT HARVESTING SCHEDULES OF FLORIDA 66 ALFALFA DURING THE 1973 GROWING SEASON

Harvesting schedule	Cuts										Mean/cut
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
3 weeks	490	667	742	856	871	884	870	866	831	676	776
6 weeks	1165	1715	1755	1736	1507	-	-	-	-	-	1575
1st flower	784	902	1074	1208	1180	1260	1150	-	-	-	1080
Full bloom	1165	1026	1444	1307	1470	1350	-	-	-	-	1294
1000 h.u.	1000	1000	1000	1000	1000	1000	1000	-	-	-	1000
1500 h.u.	1500	1500	1500	1500	1500	-	-	-	-	-	1500

TABLE 19. DRY MATTER YIELD (MT/HA) OF FLORIDA 66 ALFALFA SUBJECTED TO DIFFERENT HARVESTING SCHEDULES IN THE 1972 GROWING SEASON

Harvesting schedule	Cuts*										Total
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
3 weeks	1.65	1.56	.36	.46	.42	.16	.26	.11	0	.18	5.16d **
6 weeks	2.75	2.12	2.07	1.32	.89	-	-	-	-	-	9.19a
1st flower	1.90	1.06	1.25	1.02	.78	.67	.68	-	-	-	7.36c
Full bloom	2.48	2.25	1.70	1.30	.57	-	-	-	-	-	8.29b
1000 h.u.	2.39	1.67	1.05	1.46	.61	.45	.29	.61	-	-	8.54ab
1500 h.u.	2.61	2.00	2.24	1.27	.72	-	-	-	-	-	8.82ab

* Average of 6 replications. Staging and frost-kill cuts not included.

** Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 20. DRY MATTER YIELD (MT/HA) OF FLORIDA 66 ALFALFA SUBJECTED TO DIFFERENT HARVESTING SCHEDULES IN THE 1973 GROWING SEASON

Harvesting schedule	Cuts*									Total
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	
3 weeks	.52	.41	.34	.27	.22	.17	.06	.16	.04	2.19d**
6 weeks	2.23	2.29	1.77	1.73	.76	-	-	-	-	8.79a
1st flower	1.30	1.07	.93	.78	.56	.46	.31	-	-	5.41c
Full bloom	2.08	1.82	1.58	.99	.86	.57	-	-	-	7.89b
1000 h.u.	1.55	1.37	.87	.59	.40	.43	.36	.32	-	5.90c
1500 h.u.	2.40	2.00	1.51	1.04	.71	-	-	-	-	7.67b

* Averages of 6 replications. Staging and frost-kill cuts not included.

** Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 21. PERCENTAGE AND YIELD OF CRUDE PROTEIN (CP) IN FLORIDA 66 ALFALFA UNDER DIFFERENT HARVESTING SCHEDULES IN 1972 AND 1973

Harvesting schedule	1972		1973	
	CP, %	MT/ha CP	CP, %	MT/ha CP
3 weeks	24.0a*	1.24d	31.2a	.68d
6 weeks	18.0d	1.65b	23.9d	2.09a
1st flower	21.2c	1.55bc	24.9c	1.34c
Full bloom	17.9d	1.48c	24.2cd	1.92ab
1000 h.u.	21.6b	1.85a	26.0b	1.53bc
1500 h.u.	17.9d	1.57bc	22.7e	1.74b

*Values within each vertical column followed by the same letter are not significantly different at $P < .05$.

TABLE 22. *IN VITRO* ORGANIC MATTER DIGESTIBILITY (IVOMD) AND YIELD OF DIGESTIBLE ORGANIC MATTER (DOM) IN FLORIDA 66 ALFALFA IN 1972 AND 1973

Harvesting schedule	1972		1973	
	IVOMD %	DOM MT/ha	IVOMD %	DOM MT/ha
3 weeks	73.0a*	3.78c	74.6a	1.63d
6 weeks	64.5c	5.93a	67.2e	5.91a
1st flower	70.2b	5.17b	70.4c	3.81c
Full bloom	65.1c	5.40ab	68.2d	5.38ab
1000 h.u.	69.2b	5.90a	71.7b	4.23c
1500 h.u.	63.4c	5.59ab	67.4de	5.17b

* Values within each vertical column followed by the same letter are not significantly different at $P<.05$

TABLE 23. EFFECT OF HARVESTING SCHEDULES ON THE PERSISTENCE OF FLORIDA 66 ALFALFA IN THE 1972 AND 1973 GROWING SEASONS

Harvesting schedule	Living plants/1 m row in 1972			Living plants/1 m row in 1973		
	April	November	% survival	April	November	% survival
3 weeks	66	22	35.4e*	20	9	29.5e
6 weeks	76	41	47.3b	31	20	40.1b
1st flower	76	34	41.7d	25	17	36.3d
Full bloom	69	44	52.7a	35	27	44.4a
1000 h.u.	78	37	43.7c	30	19	38.6c
1500 h.u.	78	42	47.0bc	31	22	39.9b

* Values within each vertical column followed by the same letter are not significantly different at $P < .05$

CHAPTER VI

EXPERIMENT IV

EFFECT OF STAGE OF MATURITY ON HERBAGE YIELD AND FEEDING VALUE OF FLORIDA 66 ALFALFA

Introduction

In previous experiments with Florida 66 alfalfa it was indicated that when the alfalfa was cut at a young stage of maturity it was highly digestible, but that it did not continue to yield enough total forage to make further production economical. Moreover, at young stages of maturity, e.g., 3 weeks, alfalfa was cut more frequently than any other treatment. This means that the increased number of haying operations per season, coupled with greater difficulty in drying the immature forage, further increases the cost and labor involved with the short cutting interval. At the other extreme, it would be desirable to know when to cut the alfalfa before it is so mature that decreased digestibility offsets the increased yield obtained as the forage matures.

The results of Experiment IV being reported here have covered the DM yield and quality (chemical composition, intake, digestibility and their interrelationships) of Florida

66 alfalfa at three stages of maturity during part of the 1973 growing season.

Materials and Methods

Approximately 0.8 ha of land at the University of Florida Agronomy Farm, Gainesville, were seeded with Florida 66 alfalfa in 25.4-cm rows on October 13, 1972. A seeding rate of 22.4 kg/ha was used. Prior to seeding, dolomitic lime had been applied to the soil, the Arredondo loamy fine sand (a Grossarenic Paleudult sub-group) on September 13, 1972, at the rate of 2240 kg/ha. Just before seeding time, 48.8, 186, 11.2 and 4 kg/ha of P, K, FTE 503 and aldrin, respectively, were disked into the top soil.

On April 3, 1973, all the topgrowth was cut and removed. The area was divided into three strips, A, B and C to be cut at 3, 4.5 and 6 weeks of maturity, respectively, as shown below:

Strip	Maturity and date of cutting	Total no. of cuts
A	3 weeks 4/24, 5/15, 6/5,...	3
B	4.5 weeks 5/4, 6/6, 7/5, 8/8	4
C	6 weeks 5/16, 6/27, 8/9	3

Strips A and C received 24.4, 93 and 5.6 kg/ha of P, K and FTE 503, respectively, on March 4, April 5, May 17 and June 29. Strip B had similar amounts on March 4 and April 5, and then 36.6, 139.5 and 8.4 kg/ha of P, K and FTE 503, re-

spectively, on June 7 and July 6.

Alfalfa was cut to a 4-cm stubble using a tractor-mounted sickle-bar mower. Cutting was done in the morning and the partially wilted forage loaded onto a drying wagon in the afternoon. Drying was completed overnight, using forced heated air at about 60C. Shortly afterwards the dry hay was chopped and stored in bags until feeding time.

Percent CP, NDF and IVOMD in Field Samples

Before each cutting, samples of alfalfa forage were cut from four randomly selected areas (approx. 0.0004 ha each) in each strip, dried and ground in a Wiley mill to pass through a 1-mm screen. Later they were analysed for %CP and %IVOMD as in Experiment III, and for %NDF using the method of Van Soest (231).

In Vivo Intake and Digestibility

A total of nine hays, 2, 4 and 3 from the 3-, 4.5- and 6-week mature alfalfa, respectively, were used in the feeding trial (Table A-10). Voluntary intake and nutrient digestibility of the hays by sheep were determined, using the procedure of Moore and Walker (143). Thirty six Florida native x Suffolk wether lambs 10 months old and averaging 32.1 kg of body weight were fed the nine hays in a randomized complete block design. Sheep were assigned to four blocks according to initial weights, and randomly assigned to the nine treatments within blocks.

Sheep were fed in individual cages, 0.67 m by 1.33 m, with slatted floors and provision to collect any hay dropped on the floor of the cage ("waste"); water was available continuously through an automatic system; salt which contained micronutrients and defluorinated phosphate was kept before each animal in separate boxes. Feces were collected in canvas bags attached to the animal by means of a harness; urine was not collected.

A week prior to the experiment the sheep were confined in pens in a barn and all fed a standard hay of alfalfa plus salt and phosphate. The sheep were wormed on the first day of this conditioning period. A preliminary period of 14 days was allowed during which the amount of experimental hays offered was adjusted to achieve voluntary intake. The sheep were fed once daily at 10:00 a.m. and orts (refused hay) were weighed just before feeding. If a sheep left less than 200 g on any given day, the amount offered was increased by 300 g over the amount offered the day before. When more than 300 g were left for three consecutive days, the amount offered was reduced by 100 g each day until 200-300 g were left.

The 15th through 21st days constituted the intake measurement period. On each of these days a sample of hay was taken representing an average weight of the total amount offered to all sheep receiving that hay; each sample was placed in an airtight plastic bag. The seven daily hay samples were com-

posited, ground through a hammermill with a 0.63 cm screen, mixed and about a 2-kg subsample was reground through a Wiley mill with a 4-mm screen, mixed and a 200-g portion reground through a 1-mm screen, of which about 80 g was stored in an airtight polyethylene bag.

The total quantity of orts from each sheep was collected, weighed and saved each day from the 16th through the 22nd day. The total orts for each sheep for seven days were composited, dried in forced heated air at about 60C and allowed to equilibrate with atmospheric moisture for two days. They were then weighed and immediately ground through the 4-mm and 1-mm screens of the Wiley mill as above. A sample of about 80 g was stored in an airtight polyethylene bag.

Waste was allowed to accumulate from day 16 through 22 when a thorough collection was made. The total collection from each sheep was weighed and if it exceeded 140 g (20 g/day) it was dried, equilibrated and ground as described for orts above. Waste of less than 140 g for seven days was considered within experimental error and discarded.

Feces bags were emptied daily throughout the experiment; this was done immediately after removing orts and prior to feeding. On the 17th through 23rd days, the total quantity of feces voided during the previous 24 hours was collected in a bucket, weighed, mixed by hand and a representative 20% portion placed in a plastic bag and frozen. The seven daily feces sample from each sheep, representing

20% of the total voided, were placed in one large nylon bag and dried under forced air at 60C using a crop drier. After equilibration with atmospheric moisture, the total sample was weighed and ground through the 4-mm screen of the Wiley mill. A representative sub-sample was immediately reground through the 1-mm screen and stored in an airtight polyethylene bag.

After the experiment, dry matter (DM), organic matter (OM), CP, and NDF (ash free) analyses were carried out on the ground (1-mm) hay, orts, waste and feces samples. Hays and orts were analyzed for IVOMD.

Voluntary OM intake, digestible OM intake, and apparent OM digestibility were calculated for each sheep as follows:

Average daily OM intake = A - B - C

Average daily digestible OM intake = A - B - C - D

Apparent OM digestibility $= \frac{A - B - C - D}{A - B - C} \times 100$

Where,

A = avg. daily OM offered, g

$= \frac{(\%\ \text{OM of hay})}{100} \times \frac{(\text{total weight of hay offered})}{7}$

B = avg. daily OM refused (orts), g

$= \frac{(\%\ \text{OM of orts})}{100} \times \frac{(\text{total weight of orts refused})}{7}$

C = avg. daily OM wasted, g

$= \frac{(\%\ \text{OM of waste})}{100} \times \frac{(\text{total weight of waste})}{7}$

D = avg. daily OM excreted (feces), g

$$= \frac{(\%\ \text{OM of dried feces})}{100} \times \frac{5 \times (\text{wt. of feces aliquot})}{7}$$

Intake and digestion of CP and NDF were calculated by multiplying hay, orts, waste and feces weights by the percentages of CP and NDF (ash-free) in hay, orts, waste and feces, and substituting these values as appropriate in the above equations. After fecal collection and before feeding on the 23rd day, the sheep were weighed. This weight was used in expressing the average daily intakes in terms of g/kg $BW^{.75}$.

Percent IVOMD, % CP and % NDF of Leaf and Stem Constituents

Representative samples of chopped hay were saved from each of the nine hays that were fed to sheep. Exactly 0.5 kg portions of each were separated by hand into leaf and stem fractions. After weighing, the separate portions were ground through a 1-mm screen of a Wiley mill. Proportionate weights of the two ground leaf and stem fractions were mixed together to reconstitute a whole sample. Sub-samples of the ground leaf, stem and reconstituted whole sample, making a total of 27, were analysed for % IVOMD, % NDF and % CP. These values were then compared with those obtained on samples of whole alfalfa plants which were cut from the field at the time the hay material was cut, and also with similar values obtained on refused hay (orts).

Results and discussion

Dry Matter Yield

Dry matter yields of Florida 66 alfalfa cut at 3, 4.5 and 6 weeks of age between April 2 and August 9, 1973 are shown in Table A-9. Originally, it had been planned to obtain six cuts from the alfalfa strip that was cut at three weeks of age. However, only three cuts were made, beyond which the stand of alfalfa plants was too thin to realize sufficient hay in further cuts for the intake and digestibility study. Most of the second cut 3-week alfalfa's tender leaves and stems were pulverized by the chopper as it was chopped when it was still too dry and brittle. Therefore, this hay was not included in the intake and distibility study. The 4.5- and 6-week treatments had four and three cuts, respectively, as originally planned. Within a period of 18 weeks, cutting at 4.5 weeks of maturity produced a total of 9.0 MT/ha while the 6-week cutting interval produced a 6.9 MT/ha.

Percentages of CP, NDF and IVOMD in Hay

The percentages of CP, NDF and IVOMD of the nine hays that were chopped and fed to sheep are presented in Table 24. Percent CP ranged from 16.8 (third cut of 6-week hay) to 30.6 (first cut of 3-week hay), with a mean of 22.5. Within each maturity group CP percentage tended to decrease at each consecutive cutting. Percent NDF ranged from 33.3

(first cut of 3-week hay) to 51.7 (second cut of 6-week hay), with a mean of 43.8. Within each maturity group, NDF generally increased progressively with subsequent cuts. Percent IVOMD ranged from 59.5 (second cut of 6-week hay) to 73.9 (1st cut 3-week hay), with a mean of 65.5. Within each maturity group, subsequent cuts had lower IVOMD than the first cut.

Effect of Hay-making on CP, NDF and IVOMD

At the time each crop of hay was cut, fresh samples of whole alfalfa plants were taken and dried separately from the rest of the hay. Percentages of CP, NDF and IVOMD of these samples which did not go through the processes of hay-making and chopping are shown in Table 24 alongside those of their respective chopped hays. In all cases but one, chopped hays had lower CP than field samples, with a mean reduction of 2.0 units. By contrast, all chopped hays had higher NDF than field samples, with a mean increase of 5.0 units. Percent IVOMD in all but one case were lower in chopped hay than in field samples, with a mean reduction of 2.2 units.

Higher NDF and lower CP and IVOMD values in chopped hay were largely due to loss of dry leaves and other tender plant parts which were visibly lost during the loading of the partially sun-dried alfalfa onto the wagon where drying was completed with forced hot air, the unloading of the dry hay from the wagon into the chopper, the chopping process and

bagging and transportation to storage of the chopped hay. Chopping was only necessary for ease of handling daily amounts of hay that were fed to sheep in individual cages. Ordinarily, such losses of tender parts would occur during the baling of sun-dried hay, without further losses in the chopper.

Organic Matter Intake and Digestibility by Sheep

Results of OM intake and digestibility by sheep of Florida 66 alfalfa at three stages of maturity are presented in Table 25. Average daily total OM intake in g/kg $BW^{.75}$ ranged from 75.5 (second cut of 6-week hay) to 92.9 (second cut of 3-week hay), with a mean of 85.4. However, there was no significant difference between total OM intake of all the nine hays. Digestible OM intake (DOMI) in g/kg $BW^{.75}$ per day ranged from 46.7 (second cut of 6-week hay), which was significantly the lowest, to 66.8 (third cut of 3-week hay), which was significantly the highest intake. The first cut of 3-week alfalfa had the second highest DOMI of 62.7 which was not significantly different from that of the third cut 3-week hay. There was no definite trend in DOMI values within each of the three maturity groups. However, between maturity groups, mean DOMI decreased with increasing maturity.

The highest percent in vivo OM digestibility (OMD) was 72.2 from the first cut of 3-week hay which, together with 71.8 (third cut of 3-week hay) and 70.4 (first cut of 4.5-week hay), were significantly the highest. The lowest percent in vivo OMD value was 62.5 from the third cut of 6-hay

which, together with 63.0 (second cut of 6-week hay) and 63.7 (second cut of 4.5-week hay), were significantly the lowest. Within maturity groups, digestibilities decreased with subsequent cuts except the second cut of 4.5-week hay whose digestibility was lower than those of the two subsequent cuts. Between maturity groups, mean *in vivo* OMD decreased with increasing stage of maturity.

A positive relationship between DOMI and *in vivo* OMD of various forages has been reported by numerous workers in the literature. Using the nine hays in this experiment, this relationship was found to have the following regression equation: $Y = 34.73 + 1.37X$; $S_{y.x} = 3.41$, where X = *in vivo* OMD (%) and Y = DOMI in g/kg $BW^{.75}$. The correlation coefficient ($r = + 0.85$) was positive and significant at $P < .01$.

In Vivo - *In Vitro* Relationship

The use of an *in vitro* technique in assessing the nutritional quality of forages depends upon its accuracy and reproducibility in predicting a given animal response. The relationship between *in vivo* (sheep) and *in vitro* (two-stage) OM digestibility of Florida 66 alfalfa at three stages of maturity was found to have the following regression equation: $Y = 17.68 + .75X$; $S_{y.x} = 1.06$; $r = + .96$, where Y was the dependent variable representing percent *in vivo* OMD and X, the independent variable representing % IVOMD. There was a very high and significant ($P < .001$) positive correlation

(r = + 0.96) between the nine pairs of data. This relationship is illustrated in Figure 9.

As shown in Table 26, with the exception of first cut 3-week hay, IVOMD underestimated in vivo OM digestibility coefficients obtained with sheep by an average of -1.7 units. The sheep in these trials were fed at a 10 to 15 percent refusal level. Data presented in Table 26 show that IVOMD of the hay refused (orts) by sheep in all the nine groups were lower than IVOMD of the original hays by a mean of -12.2 percentage units. This means that the sheep exercised some degree of selection of the alfalfa hay offered, a fact that may account in part for the observed differences between in vivo and in vitro coefficients.

At the time of writing, no in vivo-in vitro digestibility relationships on alfalfa had been found in the literature. Barnes (16) found that the two-stage in vitro technique was equally effective in predicting the digestibility of both grasses and legumes, and in a collaborative study using the two-stage in vitro technique, Barnes (18) reported correlation coefficients ranging from 0.79 to 0.97 for individual laboratories, and standard errors of estimate ranging from 1.8 to 4.4, for the in vivo-in vitro digestibility relationship. Therefore, the low standard error of estimate ($S_{y.x}$ = 1.06) and the highly significant ($P < .001$) positive correlation (r = + 0.96) between in vivo-in vitro digestibility of Florida 66 alfalfa which was obtained in this study gives

additional credit to the two-stage *in vitro* technique, especially the modification by Moore et al. (144) which is in use at the Forages Laboratory, University of Florida.

Intake and Digestibility of CP by Sheep

Table 27 shows average daily intake data of total and digestible CP and its apparent digestibility in Florida 66 alfalfa. Within maturity groups, first-cut hays had higher total and digestible CP intake than subsequent cuts. Between maturity groups, intake of total and digestible CP decreased with increasing maturity. Average daily intake (g/kg $BW^{.75}$) of total CP ranged from 14.8 (second cut 6-week hay) which, together with 15.2 (third cut 6-week hay), were significantly the lowest, to 26.4 (first cut 3-week hay) which was significantly the highest. Average daily intake of digestible CP ranged from 10.4 (third cut 6-week hay) which, together with 10.6 (second cut 6-week hay), were significantly the lowest, to 21.5 (first cut 3-week hay) which was significantly the highest.

Within maturity groups, first cut hays had higher apparent digestibilities of CP, while, between maturity groups, mean apparent digestibility decreased with increasing maturity. The lowest value, 67.8%, was from the third cut of 6-week hay, while 78.4% from the first cut of 3-week hay and 78.1% from the first cut of 4.5-week hay were significantly the highest.

Reduction in composition (Table 24), intake and digestibility (Table 27) of CP with each increase in the cutting interval reported above conforms with similar reports in the literature, especially those of Weir et al. (240).

Intake and Digestibility of NDF

Average daily intake (g/kg $BW^{.75}$) of total and digestible NDF in Florida 66 alfalfa by sheep, as well as apparent digestibility of NDF, are shown in Table 28. Within each of the three maturity groups total NDF intake increased steadily at each subsequent cutting, while between groups, mean total NDF intake increased as cutting interval was lengthened. The lowest total NDF intake was 28.4, and the highest 43.4, g/kg $BW^{.75}$ from the first cut 3-week, and third cut 6-week, hay, respectively.

Within maturity groups, average daily intake of digestible NDF increased at each subsequent cutting in the 3- and 6-week groups but had no definite trend in the 4.5-week group. However, between the three maturity groups, mean intake coefficients increased as the cutting interval was lengthened.

Percentages of apparent digestibility of NDF were similar in all the three 6-week hays, in the two 3-week hays and in all the 4.5-week hays except the first cut which was lower but not significantly different from the two 3-week hays. Between groups, NDF in the 3-week hays was the most digestible, while mean NDF digestibility in the 4.5- and 6-week hays were about the same. In a similar study, Weir et al.

(240) reported no difference in fiber (crude) digestibility at the 4- and 5-week cutting intervals, but the fiber of the 6-week cutting interval was markedly less digestible than that from the 3-week cutting interval.

Relationships Among Some Components of Intake, Digestibility and Forage Composition

While it is realized that animal productivity may be related more closely to the intake of digestible energy from forages than to the apparent digestibility of dry matter or energy, and that the level of intake may not necessarily be related to digestibility (207, 230) it has nevertheless been found to be difficult to develop a laboratory procedure for the prediction of intake with acceptable accuracy. Barnes (16), for instance, has found that the standard error of estimate for the prediction of *in vivo* digestibility from *in vitro* data in temperate forages was of the order of 2 units, while the standard error for the prediction of voluntary intake from in vitro measurements was approximately 5 units.

The relationships between chemical entities of the hay and components of intake and digestibility in this study were established by regression equations shown in Table 29. There was a positive and significant correlation between CP and digestible crude protein intake (DCPI). Digestible CP intake was also positively and significantly correlated with both DOMI and percent *in vivo* OM digestibility. There was a negative and significant correlation between NDF and DOMI,

but with a larger standard error of estimate ($S_{y.x}$ = 3.80).

NDF, CP and IVOMD of Leaf and Stem Fractions

The percentage of NDF, CP and IVOMD leaf, stem and leaf + stem fractions of nine hays of Florida 66 alfalfa at three stages of maturity are presented in Table 30. The leaf to stem ratios also shown in the table show that the two 3-week hays had the highest amounts of leaves in the hay. The first cut of 4.5-week, and third cut of 6-week hays also had more leaf than stem. Between the three maturity groups, the 3-week hays had the highest mean leaf to stem ratio while mean ratios of the 4.5- and 6-week hays were similar.

The lowest leaf to stem ratio shown in the 4.5-week second cut cannot be explicitly explained. This low ratio was later reflected in the quality of the hay. It had the lowest DOMI, and one of the three lowest *in vivo* OM digestibilities (Table 25). Within the 4.5-week hays the second cut hay had the lowest CP (Table 24) and intake of digestible NDF (Table 28). Lastly, orts (hay refused by sheep) from the 4.5-week hay had the lowest IVOMD (Table 26).

Percent NDF generally increased with advancing maturity in leaf, stem, and leaf + stem material. Within each maturity group, NDF tended to increase at each subsequent cutting. This trend was more distinct in the leaf fraction. As would be expected, mean NDF was highest in stem (59.0%), moderate in leaf + stem (41.3%) and lowest in leaf (28.1%).

Crude protein percentage decreased with advancing maturity in the reverse order of NDF namely, highest in leaf (33.7%), moderate in leaf + stem (25.3%) and lowest in stem (13.7%). Within maturity groups, subsequent cuts tended to have lower CP.

Percent IVOMD generally fell as the cutting interval was lengthened. Within maturity groups subsequent cuts tended to have lower IVOMD values. Mean IVOMD was highest in the material with the lowest NDF and highest CP, and vice-versa, i.e., highest in leaf (75.2%), moderate in leaf + stem (66.1%) and lowest in stem (55.0%). The effect of NDF and CP on IVOMD was further determined by pooled regression analysis using 17 samples of Florida 66 alfalfa (six 3-week, six 4.5-week, and five 6-week, hays) collected from the same respective strips where the feeding hays were cut, between April 2 and October 30, 1973. The pooled regression equations were:

$$Y = 101.00 - .85X;\ S_{y.x} = 0.96;\ r = -.98,\ P < .001$$

$$Y_1 = 45.67 + .94X_1;\ S_{y.x} = 2.09;\ r = +.90,\ P < .001$$

where Y and Y_1 were dependent variables representing IVOMD and X and X_1 were independent variables representing NDF and CP, respectively. Thus, percent IVOMD in alfalfa can be predicted equally well by both NDF and CP, although using CP was associated with a slightly higher standard error of estimate ($S_{y.x} = 2.09$).

Data presented above are in general agreement with previous reports that leafiness in alfalfa is associated with

quality (19, 196); that the percentage of leaf tissue declines with maturity (108, 130, 148, 254); and that stems contain more fiber than leaves (108, 254). Although leafiness alone may not be adequate as a sole criterion of nutritional quality of alfalfa, quality estimates may well be improved by placing more emphasis on the leaf to stem ratio. This can be accomplished by cutting at an appropriate stage of maturity, by using cultivars resistant to leaf diseases, by pest control and by proper drying and handling of hay in order to save as much of the leaf material as possible.

TABLE 24. EFFECT OF HAY-MAKING ON CRUDE PROTEIN (CP) NEUTRAL DETERGENT FIBER (NDF) AND IN VITRO ORGANIC MATTER DIGESTION (IVOMD) OF FLORIDA 66 ALFALFA HAY

Maturity (weeks)	Harvest Date (1973)	CP, % of dry matter			NDF, % of dry matter			IVOMD, %		
		Field Samples	Chopped Hay	Diffe-rence	Field Samples	Chopped Hay	Diffe-rence	Field Samples	Chopped Hay	Diffe-rence
3	4/23	35.9	30.6	-5.3	26.4	33.3	+6.9	76.0	73.9	-2.1
3	6/5	26.6	24.9	-1.7	35.1	40.3	+5.2	72.3	70.2	-2.1
4.5	5/4	27.1	27.5	+ .4	34.0	39.9	+5.9	70.9	67.8	-3.1
4.5	6/6	22.9	21.4	-1.5	41.7	46.2	+4.5	64.5	62.3	-2.2
4.5	7/4	23.5	21.9	-1.6	39.8	43.1	+3.3	67.8	65.8	-2.0
4.5	8/6	22.8	18.9	-3.9	40.3	47.0	+6.7	66.3	62.6	-3.7
6	5/15	23.6	22.2	-1.4	40.2	41.5	+1.3	66.5	67.0	+0.5
6	6/25	19.6	18.4	-1.2	46.7	51.7	+5.0	61.7	59.5	-2.2
6	8/6	18.9	16.8	-2.1	45.0	51.1	+6.1	62.8	60.4	-2.4
Mean		24.5	22.5	-2.0	38.8	43.8	+5.0	67.6	65.5	-2.2

TABLE 25. ORGANIC MATTER INTAKE AND DIGESTIBILITY BY SHEEP OF FLORIDA 66 ALFALFA HAY CUT AT THREE STAGES OF MATURITY

Maturity (weeks)	Avg Daily Intake ($g/Kg\ BW^{.75}$) Total OM	Digestible OM	% Apparent *in vivo* OMD
3	87.0a*	62.7ab	72.2a
3	92.9a	66.8a	71.8a
4.5	81.6a	57.4cd	70.4ab
4.5	84.1a	53.6d	63.7de
4.5	90.7a	60.5bc	67.8c
4.5	83.5a	54.9cd	65.8cd
6	86.0a	58.0bcd	68.1bc
6	75.5a	46.7e	63.0e
6	87.2a	54.4cd	62.5e

* Values within each vertical column followed by the same letter are not significantly different at $P < .05$

TABLE 26. DIFFERENCES BETWEEN IN VITRO ORGANIC MATTER DIGESTION (A) AND IN VIVO ORGANIC MATTER DIGESTIBILITY (B) OF FLORIDA 66 ALFALFA HAY CUT AT THREE STAGES OF MATURITY, AND BETWEEN A AND IN VITRO ORGANIC MATTER DIGESTIBILITY OF HAY REFUSED BY SHEEP (ORTS) (C) IN THE FEEDING TRIAL

Maturity (weeks)	% Digestibility				
	A In vitro (hay)	B In vivo (hay)	Difference (A-B)	C In vitro (orts)	Difference (A-C)
3	73.9	72.2	+1.7	66.2	- 7.7
3	70.2	71.8	-1.6	59.9	-10.3
4.5	67.8	70.4	-2.6	57.7	-10.1
4.5	62.3	63.7	-1.4	47.0	-15.3
4.5	65.8	66.6	-2.0	48.9	-15.7
4.5	62.6	65.8	-3.2	47.4	-15.2
6	67.0	68.1	-1.1	52.2	-14.8
6	59.5	63.0	-3.5	49.2	-10.3
6	60.4	62.5	-2.1	51.4	- 9.0
Mean	65.5	67.2	-1.7	53.3	-12.2

TABLE 27. INTAKE AND DIGESTIBILITY BY SHEEP OF CRUDE PROTEIN (CP) IN FLORIDA 66 ALFALFA HAY CUT AT THREE STAGES OF MATURITY

Maturity (weeks)	Avg Daily Intake (g/Kg $BW^{.75}$)		% Apparent *in vivo* Digestibility of CP
	Total CP	Digestible CP	
3	26.4a*	21.5a	78.4a
3	23.0b	17.3b	75.2d
4.5	21.2c	16.6bc	78.1ab
4.5	19.5d	14.4e	73.8de
4.5	20.9cd	15.8cd	75.4cd
4.5	16.6e	12.4f	74.8de
6	20.1cd	15.4de	76.6bd
6	14.8f	10.6g	72.0e
6	15.2ef	10.4g	67.8f

* Values within each vertical column followed by the same letter(s) are not significantly different at $P < .05$

TABLE 28. INTAKE AND DIGESTIBILITY BY SHEEP OF NEUTRAL DETERGENT FIBER (NDF) IN FLORIDA 66 ALFALFA HAY CUT AT THREE STAGES OF MATURITY

Maturity (Weeks)	Avg Daily Intake ($g/Kg\ BW^{.75}$) Total NDF	Avg Daily Intake ($g/Kg\ BW^{.75}$) Digestible NDF	% Apparent *in vivo* Digestibility of NDF
3	28.4d*	17.7cd	62.7a
3	36.9b	23.8a	64.4a
4.5	31.7cd	19.2bc	60.7a
4.5	35.0bc	16.7d	47.4c
4.5	36.7b	19.0bcd	51.8bc
4.5	37.0b	18.3cd	49.4c
6	33.4bc	18.4cd	54.7c
6	37.2b	19.2bc	51.8bc
6	43.4a	22.2a	51.1bc

* Values within each vertical column followed by the same letter(s) are not significantly different at $P < .05$

TABLE 29. RELATIONSHIPS AMONG SOME PARAMETERS OF INTAKE AND DIGESTIBILITY AND FORAGE COMPOSITION

Dependent variable (Y)	Independent variable (X)	Regression equation	$S_{y.x}$	r	F
DCPI[a]	CP[b]	Y = 1.89 + .75X	1.13	.95	70.5***
DOMI[c]	DCPI[a]	Y = 37.76 + 1.30X	3.84	.79	11.35*
OMD[d]	DCPI[a]	Y = 52.87 + .96X	1.69	.90	31.95***
DOMI[c]	NDF[e]	Y = 91.54 - .78X	3.80	-.79	11.68*

[a]DCPI = digestible crude protein intake g/kg $BW^{.75}$
[b]CP = crude protein, % of dry matter
[c]DOMI = digestible organic matter intake, g/kg $BW^{.75}$
[d]OMD = organic matter digestibility
[e]NDF = neutral detergent fiber, % of dry matter
*, *** = significant at 5 and .1%, respectively
$S_{y.x}$ = standard error of estimate
r = correlation coefficient
F = variance ratio

TABLE 30. NEUTRAL DETERGENT FIBER (NDF), CRUDE PROTEIN (CP), AND *IN VITRO* ORGANIC MATTER DIGESTION (IVOMD) OF LEAF, STEM AND LEAF+STEM OF FLORIDA 66 CHOPPED HAY AT THREE STAGES OF MATURITY

Maturity (weeks)	Leaf/Stem ratio	NDF, % of dry matter			CP, % of dry matter			%IVOMD		
		Leaf	Stem	Leaf+ Stem	Leaf	Stem	Leaf+ Stem	Leaf	Stem	Leaf+ Stem
3	1.44	19.9	51.8	31.1	42.9	19.4	34.1	81.3	63.0	73.8
3	1.77	29.9	57.1	39.0	33.3	14.1	27.6	76.1	55.9	69.7
4.5	1.15	22.0	60.5	37.0	39.7	13.8	28.9	79.5	55.4	67.7
4.5	.75	24.2	61.3	43.6	35.4	13.9	24.8	77.0	52.2	65.6
4.5	.95	27.8	59.5	41.2	33.0	12.6	24.8	75.3	53.4	65.9
4.5	.96	32.4	59.0	44.4	28.7	12.7	21.8	71.8	52.8	62.9
6	.82	24.6	58.6	41.9	34.2	12.5	23.7	76.8	56.1	66.4
6	.86	32.8	61.5	46.1	30.9	11.6	21.6	71.7	51.8	62.4
6	1.24	39.4	61.0	47.2	25.2	12.8	20.5	67.1	54.8	61.7
Mean	1.10	28.1	59.0	41.3	33.7	13.7	25.3	75.2	55.0	66.1

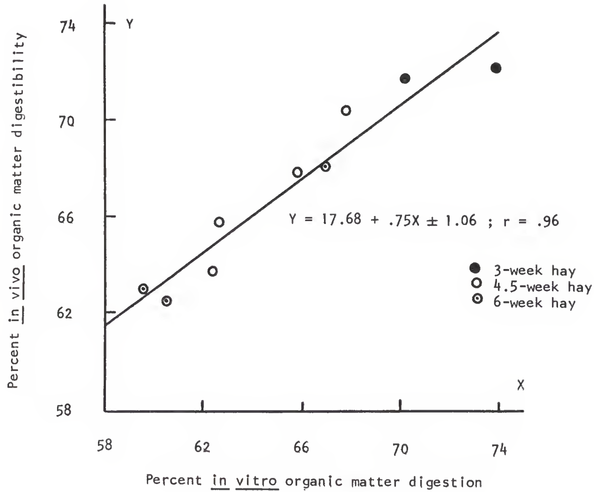


FIGURE 9. THE RELATIONSHIP BETWEEN IN VIVO AND IN VITRO ORGANIC MATTER DIGESTIBILITY OF FLORIDA 66 ALFALFA AT THREE STAGES OF MATURITY

CHAPTER VII

SUMMARY AND CONCLUSIONS

Investigations were carried out at Gainesville, Florida, on various management, morphological and quality aspects of African and Florida 66 alfalfa during the 1972 and 1973 growing seasons.

In the first experiment, carbohydrate reserves were analyzed weekly by the takadiastase enzyme method as percent total nonstructural carbohydrates (TNC, DM basis) in the roots of African and Florida 66 alfalfa which were cut at 3- and 6-week intervals. In the 1972 growing season, Florida 66 had higher TNC than African at corresponding stages of maturity, the differences being significant ($P < .05$) only at the 6-week cutting interval. Following each defoliation in the field, TNC declined for the first few weeks and then started to rise, indicating that initial regrowth of alfalfa following defoliation occurred at the expense of stored TNC.

From November 15, 1972 through October 29, 1973 root samples for TNC analysis were collected every 6 weeks from African and Florida 66 cut a 6-week interval. Florida 66 had a significantly higher mean TNC content than African at $t_{.01}$. From November 14, the TNC content of both cultivars

dropped steadily and reached their lowest levels in February. Then there was a moderate build-up leading to the first peak in April. This indicated that stored carbohydrates were utilized for respiration and maintenance during Florida's mild winter.

An alternative method of assessing food reserves in alfalfa was developed, using weekly DM yields of regrowth produced in a dark chamber by alfalfa-soil plugs. Florida 66 produced higher DM yields of regrowth than African at similar cutting intervals but these differences were not significant at $P < .05$. In all cases, the dry weight of regrowth produced in the dark chamber were proportional to their current TNC. Regression equations for the prediction of TNC using the dry weight of dark chamber regrowth showed highly positive and significant ($P < .001$) correlations between the two parameters.

A total of 10 and 5 cuts were made at the 3- and 6-week cutting intervals, respectively. However, total DM yield at the longer interval was almost twice that of the shorter interval. Florida 66 produced significantly more DM than African at both cutting intervals in 1972 and at the 6-week interval in 1973.

Morphologically, the first 15 cm of Florida 66 alfalfa's root system had a significantly greater proportion in the tap-root than African, the remaining percentage in each case being made up of branch-roots. This difference was more

pronounced in the second growing season. The length of the cutting interval had no influence on the number of stems produced by African and Florida 66. Between cultivars, African produced a significantly greater number of stems per plant than Florida 66 in both years. In 1972, 3-week alfalfa plants had a higher leaf-to-stem ratio than 6-week plants, within cultivars. Between cultivars, African had a significantly higher leaf-to-stem ratio than Florida 66 at corresponding cutting intervals. Mean leaf-to-stem ratios of 6-week African and Florida 66 were similar in 1973. At the end of each growing season, Florida 66 alfalfa plants had a higher rate of survival than African at corresponding cutting intervals. On the whole, the 3-week cutting interval progressively reduced TNC, regrowth potential, stand persistence and DM yields of both cultivars but was more severe in African.

In the second experiment, herbage DM production, persistence of stands and percent and total amounts of N, P, K, Ca, Mg, Fe, Cu, Zn and Mn in harvested Florida 66 alfalfa at different rates and frequencies of fertilization were obtained over a period of two years. In each season, a total of seven cuts were made from each of the six treatments which were cut at the first flower stage of development. Florida 66 alfalfa positively responded to fertilizer application as DM yields increased with increasing levels of fertilization up to the highest rate (97.6 and 372 kg/ha of P and K,

respectively), the differences in response between treatments being more pronounced in the second, than in the first, harvest season. Split applications of the highest two treatments produced higher yields than single applications in the second harvest season. The zero fertilizer treatment produced the least yield in both seasons. Plant persistence at the end of each growing season was enhanced by higher rates of fertilization. The zero and 24.4 and 93 kg/ha of P and K treatments had the lowest plant survival rates. The relationship between plant survival and herbage DM yields was apparently more associated with increasing fertilizer rates than with split vs single applications.

Percentages of Ca and Mg in alfalfa tops decreased with increasing amounts of fertilizer both in 1972 and 1973. These were within the range, 1 to 1.5% for Ca and .30% for Mg reported as normal compositon in alfalfa herbage DM, respectively. Amounts of Ca and Mg removed in cut herbage increased with increasing amounts of fertilizer. The levels of Fe, Zn, Cu and Mn found in cut herbage were all within the adequate range.

The percentages of N were above 3.0%, its normal composition in alfalfa, decreasing slightly with increasing fertilizer rates. The percentages of N in 1973 were higher than in corresponding treatments in 1972, but within years there was no significant difference between treatments, including

the control. Likewise, there was no significant difference between concentrations of P. All treatments, including the control, were above the adequate level of .30%, indicating that the soil of the experimental area had adequate amounts of available P for alfalfa growth. The concentration of K in, and DM yields of, alfalfa were significantly increased by increasing rates of fertilization. The zero fertilizer treatment in 1972 and 93 kg/ha of K in splits in 1973 fell below 2.0%, the adequate level of K in alfalfa. Amounts of K found in cut herbage per hectare were nearly as high as, and sometimes more than, the amount of K in applied fertilizer plus that found in 15 cm of top soil. Therefore, after meeting the basic requirements for Ca, Mg and pH through liming, and micronutrients through periodic application of fritted trace elements, K was the nutrient most likely to become limiting in alfalfa production on this soil.

In Experiment III, Florida 66 alfalfa was cut at two intervals within three different criteria, namely, fixed calendar date (3 and 6 weeks), physiological stage of maturity (first flower and full bloom) and accumulated heat units (1000 and 1500). In terms of weeks, the first flower and 1000 h.u. cutting schedules averaged 4 weeks, while the full bloom and 1500 h.u. averaged 5 and 6 weeks, respectively. In terms of heat units, the 3-week stage of maturity averaged 780 h.u,, while the first flower, full bloom and 6-week schedules averaged 1112, 1385 and 1571 h.u., respectively.

Time intervals between cuts based on flowering stages and heat units were longer during the cooler parts of the growing season, i.e., in mid-spring and the first half of fall.

The total number of cuts per treatment ranged from 5 to 10. In general, DM yields increased as the cutting interval was lengthened. In each year, the 6-week fixed cutting interval produced the highest DM yield, and the 3-week interval, the lowest. Herbage DM production in 1973 was lower than at corresponding treatments in 1972. The highest and lowest concentration of crude protein (CP) was found in the 3- and 6-week old alfalfa, respectively. However, the total yield of CP per hectare increased with increasing DM yields, being lowest where cutting intervals were shorter. The percentage of CP in 1973 were higher than those of 1972 at corresponding treatments. The yield of CP per hectare was influenced more by DM yield than by the percentage of CP in herbage. Thus, the highest and lowest 2-year yields of CP per hectare were from the 6- and 3-week cutting schedules, re-spectively. The value of alfalfa as a source of protein was demonstrated by high levels of CP at advanced stages of maturity (e.g., 18% at 6 weeks) which cannot be obtained in most other forages at comparative or lower stages of maturity.

The percentage of <u>in vitro</u> organic matter digestion (IVOMD), like CP, decreased with advancing maturity. Percentages of IVOMD in 1973 were higher than for corresponding treatments in 1972. In both years the yield of DOM was

partly influenced by IVOMD, but largely by total herbage DM yield per hectare. Thus, for the 2-year period, the highest and the lowest IVOMD, and the lowest and the highest DOM per hectare, were from 3- and 6-week old alfalfa, respectively.

In both years, the persistence of Florida 66 was generally higher where the cutting intervals were longer. The 3-week cutting interval had the lowest, while cutting at full bloom had the highest, survival rate. The 6-week interval had the second highest survival rate in both seasons. However, since the highest yield of DM, CP and DOM per hectare were from this treatment, the 6-week, or nearest interval, was considered as the best cutting schedule for Florida 66 alfalfa.

The chemical composition, intake (sheep) and digestibility (sheep cf. in vitro) of Florida 66 alfalfa at three fixed cutting intervals were studied in Experiment IV. Used in the feeding study were a total of 2, 4 and 3 hays cut at 3-, 4.5- and 6-week intervals, respectively, between April 2 and August 9, 1973. Crude protein percentage decreased at each consecutive cutting within maturity groups. Neutral detergent fiber (NDF) increased progressively with subsequent cuts. Generally, subsequent cuts were also less digestible (IVOMD) than the previous or initial cuts. In most or all cases, the nine hays which were chopped and fed to sheep had less CP, more NDF and lower IVOMD than field samples collected at similar stages of growth. This was attributed mainly to

loss of tender leaves and stems during the process of hay-making and chopping which the field samples did not go through.

Average daily total organic matter (OM) intake in g/kg $BW^{.75}$ decreased with increasing maturity, but there was no significant difference between intake of all the nine hays. However, within maturity groups, digestible OM intake (DOMI) generally decreased with subsequent cuts, while between maturity groups, mean DOMI decreased with increasing maturity. There was a positive and significant correlation ($r = + .85$) between in vivo OM digestibility (OMD) and DOMI. There was also a very high and significant ($P < .001$) and positive correlation ($r = + .96$)between IVOMD and in vivo OMD. However, the IVOMD of the same hays was on average 1.7 percentage units lower than the in vivo OMD units, due mainly to some degree of selection by sheep which were fed at a 10 to 15% refusal level.

Within maturity groups, first-cut hays had higher total and digestible CP intake and higher apparent digestibility of CP. Between the three maturity groups, intake of total and digestible CP as well a apparent digestibility of CP decreased with increasing maturity. Within maturity groups, total and digestible NDF intake increased steadily with subsequent cuts, while between groups means intake of total digestible NDF increased with advancing maturity. Apparent digestibility of NDF was generally similar within maturity groups, while between groups, NDF in 3-week hays was more digestible than in the 4.5- and 6-week hays. There were positive and

significant correlations between CP and digestible CP intake; digestible CP intake was also positively and significantly correlated with both DOMI and *in vivo* OMD. The correlation between NDF and DOMI was negative and significant.

Between maturity groups, the 3-week hays had higher mean leaf-to-stem ratios than the 4.5- and 6-week hays. Percent NDF generally increased with advancing maturity in leaf and stem fractions, being highest in the stem, moderate in the leaf + stem and lowest in the leaf. By contrast, CP was highest in the leaf, moderate in the leaf + stem and lowest in the stem. Mean IVOMD was highest in leaf, moderate in leaf + stem and lowest in stem. Thus, the quality of alfalfa may well be improved by placing more emphasis on the leaf to stem ratio, which can be accomplished by cutting at an appropriate stage of maturity, by pest and disease control, and by proper drying and handling of the hay in order to save as much of the hay as possible.

These results, therefore, support and justify the following conclusions:

(i) The greater persistence of Florida 66 over African alfalfa may partly be due to higher levels of carbohydrate reserves stored in its roots, especially under long intervals between cuts. A more developed tap-root system found in Florida 66 may also afford the plants better means of survival through anchorage and access to water and nutrients from greater soil depths.

(ii) Carbohydrate food reserves in alfalfa are estimable by the dry weight of regrowth in the dark, the two of which were found to be highly and positively correlated.

(iii) Initial regrowth of alfalfa in the field following each defoliation apparently occurred at the expense of stored TNC. By inference, therefore, the potential for Florida 66 to produce more DM than African was partly derived from its higher TNC reserve.

(iv) During Florida's mild winter, stored TNC were evidently utilized by African and Florida 66 alfalfa for respiration and maintenance through a period of little or no growth.

(v) Shorter cutting intervals (3-week) severely and progressively reduced TNC, stand persistence and DM yields of both African and Florida 66 alfalfa.

(vi) After meeting the basic requirements for pH, Ca and Mg through liming, and the micronutrients through periodic application of FTE, DM production and persistence of Florida 66 were enhanced by K fertilization up to the highest rate used (372 kg/ha/year). Response to split applications at higher rates of fertilization was only evident in the second season.

(vii) At early stages of maturity, alfalfa hay was low in NDF, high in CP and digestibility, but was low in CP and DOM yield per hectare. The highest yield of DM, CP and DOM per hectare were obtained from a 6-week, or nearest, cutting interval.

(viii) Chopped hay contained less CP, more NDF and was less digestible (IVOMD) than alfalfa field samples mainly due to loss of tender parts during the entire process of hay making and handling.

(ix) Second season alfalfa had more CP and IVOMD but less DM yield per hectare. At the same cutting interval within a season, subsequent cuts tended to have more NDF and lower DOMI, total and digestible CP intake and digestibility. Between maturity groups, DOMI, total and digestible CP and NDF intake, and nutrient digestibilities diminished with increasing maturity.

(x) Younger alfalfa had higher leaf-to-stem ratios than older alfalfa. The leaf fraction had higher CP, IVOMD and lower NDF than the stem fraction; leaf + stem having intermediate values.

(xi) The relationship between *in vivo* and *in vitro* OM digestibility was positive and highly significant. Slight underestimation of *in vivo* OMD by IVOMD was unavoidably due to some degree of selection by sheep of the hay offered.

(xii) Digestible OM intake and *in vivo* OMD of the hay were highly and positively correlated with CP and DCPI. The value of alfalfa as an imminent source of protein for human consumption and livestock feed supplements was further demonstrated by high levels of CP even at advanced stages of maturity.

APPENDIX

TABLE A-1. AVERAGE PH IN THE FIRST 15 CM OF SOIL UNDER DIFFERENT FERTILIZER TREATMENTS AT THE BEGINNING AND END OF THE 1972 AND 1973 GROWING SEASONS

Treatment	Kg/ha/yr		Number of applications	pH			
				1972		1973	
	P	K		Apr. 22	Dec. 2	Apr. 5	Nov.2
1	0	0	-	6.6	6.6	6.6	6.3
2	48.8	186	1 in spring	6.6	6.6	6.6	6.3
3	97.6	372	1 in spring	6.7	6.6	6.6	6.3
4	24.4	93	4 equal splits	6.7	6.7	6.6	6.3
5	48.8	186	4 equal splits	6.7	6.6	6.6	6.2
6	97.6	372	4 equal splits	6.8	6.7	6.6	6.4

TABLE A-2. AMOUNTS OF CALCIUM (KG/HA) FOUND IN THE 0 TO 15 CM SOIL DEPTH UNDER DIFFERENT FERTILIZER TREATMENTS AT THE BEGINNING AND END OF THE 1972 AND 1973 GROWING SEASONS

Treatment	Kg/ha/yr		Number of applications	1972		1973	
	P	K		Apr. 22	Dec. 2	Apr. 5	Nov. 2
				Ca (Kg/ha)			
1	0	0	-	854	866	987	873
2	48.8	186	1 in spring	860	817	960	892
3	97.6	372	1 in spring	955	805	979	836
4	24.4	93	4 equal splits	916	848	1004	979
5	48.8	186	4 equal splits	1004	873	991	1004
6	97.6	372	4 equal splits	960	817	1044	868

TABLE A-3. AMOUNTS OF MAGNESIUM (KG/HA) FOUND IN THE FIRST 15 CM OF SOIL UNDER DIFFERENT FERTILIZER TREATMENTS AT THE BEGINNING AND END OF THE 1972 AND 1973 GROWING SEASONS

Treatment	Kg/ha/yr		Number of applications	1972		1973	
	P	K		Apr. 22	Dec. 2	Apr. 5	Nov. 3
				Mg (Kg/ha)			
1	0	0	-	185	275	188	157
2	48.8	186	1 in spring	188	196	136	113
3	97.6	372	1 in spring	223	196	168	100
4	24.4	93	4 equal splits	205	226	162	126
5	48.8	186	4 equal splits	233	200	144	94
6	97.6	372	4 equal splits	223	140	130	88

TABLE A-4. AMOUNT OF PHOSPHORUS FOUND IN FLORIDA 66 ALFALFA HERBAGE AND IN THE SURFACE 15 CM OF SOIL UNDER DIFFERENT FERTILIZER RATES IN 1972

Treatment	Fertilizer P		Extractable soil P, 1972*		Alfalfa herbage P
	Applications No.	Amount kg/ha	April 22	December 2	
			kg/ha		
1	-	-	22.5	17.6	23.9
2	1 in spring	48.8	21.5	19.1	26.8
3	1 in spring	97.6	22.0	20.6	28.2
4	4 equal splits	24.4	21.5	17.6	26.1
5	4 equal splits	48.8	23.0	18.1	28.5
6	4 equal splits	97.6	21.5	20.1	27.2

* Extractable with ammonium acetate (pH 4.8).

TABLE A-5. AMOUNT OF PHOSPHORUS FOUND IN FLORIDA 66 ALFALFA HERBAGE AND IN THE SURFACE 15 CM OF SOIL UNDER DIFFERENT FERTILIZER RATES IN 1973

Treatment	Fertilizer P		Extractable soil P, 1973*		Alfalfa herbage P
	Applications No.	Amount kg/ha	April 5	November 2	
			kg/ha		
1	-	-	23.0	14.7	9.3
2	1 in spring	48.8	24.5	18.6	21.6
3	1 in spring	97.6	25.0	20.1	25.3
4	4 equal splits	24.4	24.0	18.1	16.9
5	4 equal splits	48.8	25.4	21.5	25.0
6	4 equal splits	97.6	31.3	26.4	29.7

* Extractable with ammonium acetate (pH 4.8).

TABLE A-6. AMOUNTS OF POTASSIUM FOUND IN FLORIDA 66 ALFALFA HERBAGE DM AND IN THE FIRST 15 CM OF SOIL UNDER DIFFERENT FERTILIZER RATES IN 1972

Treatment	Number of applications	K in Kg/ha in 1972			
		Total applied	In soil Apr. 22	In soil Dec. 2	In Alfalfa tops
1	-	-	109	110	119
2	1 in spring	186	101	158	209
3	1 in spring	372	103	199	227
4	4 equal splits	93	69	163	165
5	4 equal splits	186	76	270	191
6	4 equal splits	372	91	434	222

TABLE A-7. AMOUNT OF POTASSIUM FOUND IN FLORIDA 66 ALFALFA HERBAGE DM AND IN THE FIRST 15 CM OF SOIL UNDER DIFFERENT FERTILIZER RATES IN 1973

Treatment	Number of applications	K in Kg/ha in 1973			
		Total applied	In soil Apr. 5	In soil Nov. 2	In alfalfa tops
1	-	-	147	100	45
2	1 in spring	186	214	227	156
3	1 in spring	372	173	302	206
4	4 equal splits	93	211	213	95
5	4 equal splits	186	259	279	171
6	4 equal splits	372	336	359	244

TABLE A-9. RAINFALL AND TEMPERATURE RECORDS AT GAINESVILLE METEOROLOGICAL STATION 2 WSW

	Average Air (5') Temperature (Fahrenheit)						Rainfall (mm)		
	1972		1973		60-year				
	Max.	Min.	Max.	Min.	Max.	Min.	1972	1973	60-Year Mean
January	74.6	53.2	70.2	46.9	68.7	46.9	126	105	66
February	69.3	45.6	67.8	43.2	70.8	48.0	84	110	83
March	77.9	50.8	77.9	50.8	76.0	52.8	190	156	104
April	81.6	57.3	79.6	54.8	81.4	57.8	73	145	94
May	84.8	64.5	87.5	63.1	87.3	63.7	233	100	88
June	89.0	68.9	90.6	70.5	90.3	71.0	80	170	168
July	92.7	71.0	91.9	72.3	90.5	71.0	80	159	190
August	91.8	70.9	91.6	71.4	90.9	71.3	329	74	195
September	91.6	67.9	89.8	71.9	88.8	69.5	28	97	125
October	85.4	62.0	83.8	60.8	82.6	61.4	25	129	113
November	74.8	54.6	80.7	54.8	74.6	51.8	184	21	44
December	74.0	51.2	69.9	44.5	68.8	47.1	105	130	64

TABLE A-10. DRY MATTER YIELD OF FLORIDA 66 AT THREE STAGES OF MATURITY BETWEEN APRIL 2 AND AUGUST 9, 1973

Maturity (weeks)	Cuts			
	1st	2nd	3rd	4th
	MT/ha			
3	1.20	.96[a]	.74	-
4.5	2.12	2.12	2.02	2.72
6	2.53	2.04	2.33	-

[a]The second cut of 3-week hay was dropped from the feeding trial

BIBLIOGRAPHY

1. Adegbola, A. 1966. Preliminary observations on the reserve carbohydrates and regrowth potential of tropical grasses. Proc. 10th Intern. Grassld. Congr., Helsinki, Finland. p. 933.

2. Ademosum, A. A., B. R. Baumgardt, and J. M. Scholl. 1968. Evaluation of a sorghum-sudangrass hybrid at varying stages of maturity on the basis of intake, digestibility and chemical composition. J. Anim. Sci. 27: 818-823.

3. Akazawa, T. 1965. Starch, inulin, and other reserve polysaccharides. In Plant Biochemistry. James Bonner, and J. E. Varner (Ed.). Academic Press, N.Y. pp. 258-297.

4. Alway, F. J., and G. H. Nesom. 1945. Influence of phosphorus deficiency of the soil on the protein content of alfalfa. Amer. Soc. Agron. J. 37: 555-569.

5. American Potash Institute. July-August, 1965. Mid-West Newsletter M-134. Cited by C. L. Rhykerd, and C. J. Overdahl (173) loc. cit.

6. Anderson, M. J. 1967. Effect of harvest date, crop, and variety on the digestibility of alfalfa hays. J. Dairy Sci. 50: 990. Abstr.

7. Anderson, M. J., and D. R. Thacker. 1970. Elevation effects on nutritive characteristics of alfalfa. J.Dairy Sci. 53: 676. Abstr.

8. Anderson, M. J., G. F. Fries, D. V. Kopland, and D. R. Waldo. 1973. Effect of cutting date on digestibility and intake of irrigated first-crop alfalfa hay. Agron. J. 65: 357-360.

9. A. O. A. C. 1965. Official Methods of Analysis (8th Edn.). Association of Official Agricultural Chemists, Washington, D.C.

10. App, B. A., and G. R. Manglitz. 1972. Insects and related pests. pp. 527-550. In Alfalfa Science and Technology. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

11. Arnold, C. Y. 1959. The determination and significance of the base temperature in a linear heat unit system. Proc. Amer. Soc. Hort. Sci. 74: 430-445.

12. Balch, C. C., and R. C. Campling. 1962. Regulation of voluntary intake in ruminants. Nutr. Abstr. and Rev. 32: 669-686.

13. Balwani, T. L., R. R. Johnson, K. E. McClure, and B. A. Dehority. 1969. Evaluation of greenchop and ensiled sorghums, corn silage and perennial forages using digestion trials and VFA production in sheep. J. Anim. Sci. 28: 90-97.

14. Barber, S. A. 1963. Rainfall and response to potassium. Better Crops with Plant Food. 47 (1): 6-9.

15. Barber, S. A., and R. P. Humbert. 1963. Advances in knowledge of potassium relationships in the soil and plant. pp. 231-268. In Fertilizer Technology and Usage. M. H. Vickar, G. L. Bridger, and L. B. Nelson (Eds.). Soil Sci. Soc. Amer., Madison, Wisconsin.

16. Barnes, R. F. 1966. The development and application of in vitro rumen fermentation techniques. Proc. 10th Intern. Grassld. Congr., Helsinki, pp. 434-438.

17. Barnes, R. F. 1967. Collaborative in vitro rumen fermentation studies on forage substrates. J. Anim. Sci. 26: 1120-1130.

18. Barnes, R. F. 1969. Collaborative research with the two-stage in vitro technique. Proc. Natl. Symp. Forage Quality and Utilization. Lincoln, Nebraska. pp. N-1 to N-20.

19. Barnes, R. F., and C. H. Gordon. 1972. Feeding value and on-farm feeding. pp. 601-630. In Alfalfa Science and Technology. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

20. Barnes, R. F., and G. O. Mott. 1966. In vitro digestibility of alfalfa and reed canarygrass plant parts. Agron. Abstr. p. 40.

21. Barnes, R. F., and G. O. Mott. 1970. Evaluation of selected clones of Phalaris arundinacea L. I. In vivo digestibility and intake. Agron. J. 62: 719-722.

22. Baumgardt, B. R. 1967. Efficiency of nutrient utilization for milk production: Nutritional and physiological aspects. J. Anim. Sci. 1186-1194.

23. Baumgardt, B. R., and Dale Smith. 1962. Changes in the estimated nutritive value of the herbage of alfalfa, medium red clover, ladino clover, and bromegrass due to stage of maturity and year. Wis. Agric. Expt. Sta. Res. Rpt. 10. 17 pp.

24. Bear, F. E., and A. Wallace. 1950. Alfalfa:Its mineral requirements and chemical composition. N. J. Agric. Expt. Sta. Bull. 748. 32 pp.

25. Beardsley, A. E., and S. R. Anderson. 1960. The effect of height and frequency of cutting various strains of birdsfoot trefoil-timothy associations as compared to a Ranger alfalfa-timothy association. Agron. Abstr. p. 62.

26. Bentley, J. G., M. Moinuddin, T. V. Hershberger, E. W. Klosterman, and A. L. Mixon. 1954. Effect of trace minerals on growth performance and vitamin B_{12} synthesis of steers. J. Anim. Sci. 13: 789-801.

27. Bezeau, L. M., and L. G. Sonmor. 1964. The influence of levels of irrigation on the nutritive value of alfalfa. Can. J. Plant Sci. 44: 505-508.

28. Blaser, R. E. 1964. Symposium on forage utilization: Effect of fertility levels and stage of maturity on forage nutritive value. J. Anim. Sci. 23: 246-253.

29. Blaser, R. E. and E. L. Kimbrough. 1968. Potassium nutrition of forage crops with perennials. pp. 423-445. In The Role of Potassium in Agriculture. V. J. Kilmer, S. E. Younts, and N. C. Brady (Eds.). ASA, CSSA, SSSA, Madison, Wisconsin.

30. Blaxter, K. L., and R. S. Wilson. 1962. The voluntary intake of roughages by steers. Animal Prod. 4: 351-358.

31. Blaxter, K. L., and R. S. Wilson. 1963. The assessment of a crop husbandry technique in terms of animal production. Animal Prod. 5: 27-42.

32. Blaxter, K. L., F. W. Wainman, and R. S. Wilson. 1961. The regulation of food intake by sheep. Animal Prod. 3: 51-62.

33. Bolton, J. L. 1962. Alfalfa Botany, Cultivation and Utilization. World Crop Books, Leonard, London. 474 pp.

34. Bolton, J. L., B. P. Goplen, and H. Baenziger. 1972. World distribution and historical developments. pp. 1-34. In Alfalfa Science and Technology. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

35. Bosman, M. S. M. 1967. Jaarb. Inst. Biol. Scheik. Onderz. Landb Gewass. pp. 97-100. Cited by W. F. Raymond (168) loc. cit.

36. Bourget, S. J., and R. B. Carson. 1962. Effect of soil moisture stress on yield, water-use efficiency and mineral composition of oats and alfalfa grown at two fertility levels. Can. J. Soil Sci. 42: 7-12.

37. Briggs, P. K., J. P. Hogan, and R. L. Reid. 1957. The effect of volatile fatty acids, lactic acid, and ammonia on rumen pH in sheep. Aust. J. Agric. Res. 8: 674-690.

38. Brown, B. A. 1961. Fertilizer experiments with alfalfa (1915-1960). Conn. Agric. Expt. Sta. Bull. 363. 41 pp.

39. Brown, L. D. 1966. Influence of intake on feed utilization. J. Dairy Sci. 49: 223-230.

40. Brown, R. H., and R. E. Blaser. 1968. Leaf area index in pasture growth. Herb. Abstr. 38: 1-9.

41. Brown, R. H., R. B. Cooper, and R. E. Blaser. 1966. Effects of leaf age on efficiency. Crop Sci. 6: 206-209.

42. Buker, R. J. 1969. Higher alfalfa yields mean P-K removal. Better Crops with Plant Food. 53 (4): 6-7.

43. Bula, R. J., and Dale Smith. 1954. Cold resistance and chemical composition in overwintering alfalfa, red clover, and sweet clover. Agron. J. 46: 397-401.

44. Buller, R. E., and D. A. Sanchez. 1960. Effect of the maturity of alfalfa at harvest on forage production and stand in the valley of Mexico. Agron. Abstr. p. 62.

45. Burton, J. C. 1972. Nodulation and symbiotic nitrogen fixation. pp. 229-282. In Alfalfa Science and Technology. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

46. Burton, G. W., and J. E. Jackson. 1962. A method for measuring sod reserves. Agron. J. 54: 53-55.

47. Byers, J. H., and L. E. Ormiston. 1962. Nutritive value of forages. II. The influence of two-stages of development of an alfalfa-bromegrass hay on consumption and milk production. T. Dairy Sci. 45: 693. Abstr.

48. Calder, F. W., and L. B. McLeod. 1968. In vitro digestibility of forage species as affected by fertilizer application, stage of development and harvest dates. Can. J. Plant Sci. 48: 17-24.

49. Campling, R. C., and J. C. Murdoch. 1966. The effect of concentrates on the voluntary intake of roughages by cows. J. Dairy Res. 33: 1-11.

50. Carmer, S.G., and J. A. Jacobs. 1963. Establishment and late summer alfalfa seedings as influenced by placement of seed and phosphate fertilizer, seeding rate, and row spacing. Agron. J. 55: 28-30.

51. Chandler, R. F., M. Peach, and R. Bradfield. 1945. A study of techniques for predicting the K and Bo requirements of alfalfa: I. The influence of muriate of potash and borax on yield, deficiency symptoms and K content of plant and soil. Soil Sci. Soc. Amer. Proc. 10: 141-146.

52. Christiansen, W. C., J. Eggleston, L. R. McDowell, J. H. Conrad, and L. E. Harris. 1972. Latin American Tables of Feed Composition. Dept. Anim. Sci., and Center for Trop. Agric., University of Florida, Gainesville.

53. Coffindaffer, B. L., and O. J. Burger. 1958. Response of alfalfa varieties to daylength. Agron. J. 50: 389-392.

54. Conrad, H. R. 1966. Symposium on factors influencing the voluntary intake of herbage by ruminants: Physiological and physical factors limiting feed intake. J.Anim. Sci. 25: 227-235.

55. Conrad, H. R., A. D. Pratt, and J. W. Hibbs. 1961. Cutting date determines forage quality. Ohio Farm and Home Research. 46: 39.

56. Conrad, H. R., A. D. Pratt, and J. W. Hibbs. 1964. Regulation of feed intake in dairy cows: I. Changes in importance of physical and physiological factors with increasing digestibility. J. Dairy Sci. 47: 54-62.

57. Conrad, H. R., A. D. Pratt, J. W. Hibbs, and R. R. Davis. 1962. Relationship between forage growth stage, digestibility, nutrient intake and milk production in dairy cows. Ohio Agr. Expt. Sta. Bull. 914. 24 pp.

58. Crampton, E. W., E. Donefer, and L. E. Lloyd. 1960. A nutritive value index for forages. Proc. 8th Intern. Grassld. Congr., SaoPaulo, Brazil. pp. 462-466.

59. Crowder, L. V., J. Venegas, and J. Silva. 1960. The influence of cutting interval on alfalfa production in the high Andes. Agron. J. 52: 128-130.

60. Davies, A. 1965. Carbohydrate levels and regrowth in perennial rye-grass. J. Agric. Sci. 65: 213-221.

61. Davis, R. R., and J. L. Parsons. 1961. The effect of length of rest period and length of harvest period on yield and survival of forage crops. Ohio Agric. Expt. Sta. Res. Cir. 99. 23 pp.

62. Dijkstra, N. D. 1966. Estimation of the nutritive value of fresh roughage. 10th Intern. Grassld. Congr., Helsinki. pp. 393-397.

63. Dionne, J. L. 1965. The effect of phosphate and potash fertilizers on the botanical and chemical composition of alfalfa-timothy associatoin. Can. J. Plant Sci. 45: 18-26.

64. Doll, E. C., A. L. Hatfield, and J. R. Todd. 1959. Effect of rate and frequency of potash additions on pasture yield and potassium uptake. Agron. J. 51: 27-29.

65. Doll, E. C., A. L. Hatfield, and J. R. Todd. 1959. Vertical distribution of topdressed fertilizer phosphorus and potassium in relation to yield and composition of pasture herbage. Agron. J. 51: 645-648.

66. Dovrat, A., and Y. Cohen. 1970. Regrowth potential of rhodes grass (Chloris gayana Kunth.) as affected by nitrogen and defoliation. Proc. 11th Intern. Grassld. Congr., Surfers Paradise, Queensland, Australia. pp. 552-554.

67. Dow, A. I. 1970. Fertilizing for maximum yields of irrigated alfalfa. Wash. Coop. Ext. Serv. E. M. 3422. 7 pp.

68. Drake, M., W. G. Colby, H. Oohara, N. Yoshida, K. Fukunaga, and Y. Oohara. 1970. Perennial forage requires plenty of fertilizer in Japan. Better Crops with Plant Food. 54 (4): 22-25.

69. Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

70. Engels, E. A. N., and F. J. van der Merwe. 1967. Application of an _in vitro_ technique to South African forages with special reference to the effect of certain factors on the results. S. Afr. J. Agric. Sci. 10: 983-995.

71. Evans, J. L., J. Arroyo-Aguilu, M. W. Taylor, and C. H. Ramage. 1965. Date of harvest of New Jersey forages as related to the nutrition of ruminant animals. N. J. Agric. Expt. Sta. Bull. 814. 16 pp.

72. Feltner, K. C., and M. A. Massengale. 1965. Influence of temperature and harvest management on growth, level of carbohydrates in the roots and survival of alfalfa (_Medicago sativa_ L.). Crop Sci. 5: 585-588.

73. Ferrer, M., and R. F. Barnes. 1967. _In vitro_ digestibility of alfalfa and tall fescue plant components at different maturity stages. Agron. Abstr. p. 43.

74. Flessel, J. K., and H. D. Niemczyk. 1961. Theoretical values of fully grown first-cutting alfalfa lost to alfalfa weevil larvae. J. Econ. Entomol. 64: 328-329.

75. Fox, L. R., and R. C. Lipps. 1964. A comparison of stable strontium and P^{32} as tracers for estimating alfalfa root activity. Plant and Soil 20: 337-350.

76. Fuess, F. W., and M. B. Tesar. 1968. Photosynthetic efficiency, yields, and leaf loss in alfalfa. Crop Sci. 8: 159-163.

77. Garza, R. T., R. F. Barnes, G. O. Mott and C. L. Rhykerd. 1965. Influence of light intensity, temperature, and growing period on the growth, chemical composition and digestibility of Culver and Tanverde alfalfa seedlings. Agron. J. 57: 417-420.

78. Gerwig, J. L., and G. H. Ahlgren. 1958. The effect of different fertility levels on yield, persistence, and chemical composition of alfalfa. Agron. J. 50: 291.294.

79. Gifford, R. O., and E. H. Jansen. 1967. Some effects of soil moisture regimes and bulk density on forage quality in the greenhouse. Agron. J. 59: 75-77.

80. Gordon, C. H., J. C. Derbyshire, H. G. Wiseman, E. A. Kane, and C. G. Melin. 1961. Preservation and feeding value of alfalfa stored as hay, haylage, and direct-cut silage. J. Dairy Sci. 44: 1299-1311.

81. Graber, L. F., N. T. Nelson, W. A. Leukel, and W. B. Albert. 1927. Organic food reserves in relation to the growth of alfalfa and other perennial herbaceous plants. Wis. Agric. Expt. Sta. Res. Bull. 80. 128 pp.

82. Graham, J. H., K. W. Kreitlow, and L. R. Faulkner. 1972. Diseases. pp. 497-526. In _Alfalfa Science and Technology_. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

83. Grandfield,C. O. 1935. The trend of organic food reserves in alfalfa roots as affected by cutting practices. J. Agr. Res. 50: 697-709.

84. Graumann, H. O., J. E. Webster, C. L. Canode, and H. F. Murphy. 1954. The effect of harvest practices on the performance of alfalfa. Okla. Agric. Expt. Sta. Bull. B-433. 57 pp.

85. Greenfield, P. L., and Dale Smith. 1973. Influence of temperature change at bud on composition of alfalfa at first flower. Agron. J. 65: 871-874.

86. Groskopp, M. D., J. M. Sund, and J. T. Murdock. 1963. Irrigated alfalfa in central Wisconsin. Wisc. Afric. Expt. Sta. Bull. 558. 7 pp.

87. Grotelueschen, R. D., and Dale Smith. 1967. Determination and identification of non-structural carbohydrates removed from grass and legume tissue by various sulfuric acid concentrations, takadiastase, and water. J. Agric. Food Chem. 15: 1048-1051.

88. Grove Jr., A. R. and G. E. Carlson. 1972. Morphology and anatomy. pp. 103-122. In _Alfalfa Science and Technology_. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

89. Hanson, C. H., and R. L. Davis. 1972. Highlights in the United States. pp. 35-51. In Alfalfa Science and Technology. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

90. Hanson, R. G., and J. M. MacGregor. 1966. Soil and alfalfa plant characteristics as affected by a decade of fertilization. Agron. J. 58: 3-5.

91. Hardison, W. A., W. N. Linkous, and C. Y. Ward. 1957. Digestibility of the top and bottom portions of the alfalfa plant, as estimated from small, randomly collected samples of feces. J. Dairy Sci. 40: 768-773.

92. Hildebrand, S. C., and C. M. Harrison. 1939. The effect of height and frequency of cutting alfalfa upon consequent top growth and root development. Amer. Soc. Agron. J. 31: 790-799.

93. Hoff, J. C., and A. D. Dotzenko. 1968. Performance of alfalfa varieties under different phosphate fertilizer levels. Colorado Agric. Expt. Sta. Bull. 533-S. 12 pp.

94. Horner, E. S. 1969. Florida 66 alfalfa. An improved variety for well drained soils in Florida. Fla. Agric. Expt. Sta. Circ. S-191. 10 pp.

95. Horrocks, R. D., and J. B. Washko. 1968. Influence of harvesting forages at three stages of maturity on yield, quality, and stand persistence. Penn. Agric. Expt. Sta. Bull. 753. 22 pp.

96. Houston, C. E. 1955. Consumptive use of water by alfalfa in western Nevada. Nev. Agric. Expt. Sta. Bull. 191. 20 pp.

97. Hutton, J. B. 1963. The effect of lactation on intake in the dairy cow. Proc. N. Z. Soc. Anim. Prod. 23: 39-51.

98. Ingalls, J. R., J. W. Thomas, E. J. Benne, and M. Tesar. 1965. Comparative response of wether lambs to several cuttings of alfalfa, birdsfoot trefoil, bromegrass, and reed canarygrass. J. Anim. Sci. 24: 1159-1164.

99. Jackobs, J. A., T. R. Peck, and W.M. Walker. 1970. Efficiency of fertilizer topdressing on alfalfa. Ill. Agric. Expt. Sta. Bull. 738. 22 pp.

100. Jackson, T. L., and J. T. McDermid. 1963. Effect of method of phosphorus application on alfalfa grown on a Willamette valley "red hill" soil. Oreg. Agric. Expt. Sta. Techn. Bull. 74. 31 pp.

101. Jensen, E. H., M. A. Massengale, and D. O. Chilcote. 1967. Environmental effects on growth and quality of alfalfa. Western Regional Res. Publ. (Proj. W-58) T-9. Nevada Agric. Expt. Sta. 36 pp.

102. Johnson, R. R., and B. A. Dehority. 1968. A comparison of several laboratory techniques to predict digestibility and intake of forages. J. Anim. Sci. 27: 1738-1742.

103. Johnson, R. R., B. A. Dehority, J. L. Parsons and H. W. Scott. 1962. Descrepanses between grasses and alfalfa when estimating nutritive value from _in vitro_ cellulose digestibility by rumen micro-organisms. J. Anim. Sci. 21: 892-896.

104. Johnson, R. R., O. G. Bentley, J. W. Hibbs, and H. R. Conrad. 1956. _In vivo_ and _in vitro_ nutritional requirements of rumen microorganisms. Agric. and Food Chem. 4: 627-631.

105. Jones, R. G., F. P. Zscheile, and R. B. Griffith. 1953. Carotene and protein contents of alfalfa as influenced by variety and certain environmental factors. Hilgardia 22: 179-202.

106. Jung, G. A., and K. L. Larson. 1972. Cold, drought and heat tolerance. pp. 185-209. In _Alfalfa Science and Technology._ C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

107. Jung, G. A., and Dale Smith. 1961. Trends of cold resistance and chemical changes over winter in the roots and crowns of alfalfa and medium red clover. I. Changes in certain nitrogen and carbohydrate fractions. Agron. J. 53: 359-364.

108. Jung, G. A.,R. L. Reid, and J. A. Balasko. 1969. Studies on yield, management, persistence, and nutritive value of alfalfa in West Virginia. W. V. Agric. Expt. Sta. Bull. 581-T. 80 pp.

109. Kay, M., R. P. Andrews, N. A. MacLeod, and T. Walker. 1968. Urea and cereals as supplements for ruminants offered barley straw. Animal Prod. 10: 171-175.

110. Kehr, W. R., R. L. Ogden, and S. D. Kindler. 1970. Diallel analyses of potato leafhopper injury to alfalfa. Crop Sci. 10: 584-586.

111. Kendall, W. A. 1972. Private communication.

112. Keoghan, J. M. 1967. Effects of cutting frequency and height on topgrowth of pure lucerne stands. pp. 117-128. In The Lucerne Crop. R. H. M. Langer (Ed.). Reed, New Zealand.

113. Kiesselbach, T. A., J. C. Russel, and A. Anderson. 1929. The significance of subsoil moisture in alfalfa production. Amer. Soc. Agron. J. 21: 241-268.

114. Kimbrough, E. L. 1973. Fast alfalfa regrowth depends on potassium. Better Crops with Plant Food. 56 (4): 26-27.

115. Kimbrough, E. L., R. E. Blaser, and D. D. Wolf. 1971. Potassium effects on regrowth of alfalfa. Agron. J. 63: 836-839.

116. Klebesadel, L. J., and J. C. Brinsmade. 1966. Response of two alfalfas (Medicago sativa L. and M. falcata L.) to time and rate of potassium application in the subarctic. Agron. J. 58: 545-549.

117. Kliewer, W. M., and W. K. Kennedy. 1960. Studies on response of legumes to molybdenum and lime fertilization on Mardin silt loam soil. Soil Sci. Soc. Amer. Proc. 24: 377-380.

118. Klinkowski, M. 1933. Lucerne: Its ecological position and distribution in the world. Imperial Bureau of Plant Genetics, Aberystwyth, Wales. Herbage Plants Bull. # 12: 1-63.

119. Kresge, C. B., and S. E. Younts. 1962. Effects of various rates and frequencies of potassium application on yield and chemical composition of alfalfa and alfalfa-orchardgrass. Agron. J. 54: 313-316.

120. Kust, C. A., and Dale Smith. 1961. Influence of harvest management on level of carbohydrate reserves, longevity of stands and yields of hay and protein from Vernal alfalfa. Crop Sci. 1: 267-269.

121. Langer, R. H. M., and T. D. Steink. 1965. Growth of lucerne in response to height and frequency of defoliation. J. Agric. Sci. Camb. 64: 291.294.

122. Lassiter, C. A., R. S. Emery, and C. W. Duncan. 1958. Effect of alfalfa ash and valeric acids on growth of dairy heifers. J. Dairy Sci. 41: 552.

123. Leach, G. J. 1968. The growth of the lucerne plant after cutting: The effects of cutting at different stages of maturity and at different intensities. Aust. J. Agric. Res. 19: 517-530.

124. LeClerg, E. L., W. H. Leonard, and A. G. Clark. 1963. Field Plot Technique. Burgess Publishing Co., Minneapolis, Minnesota. 373 pp.

125. Levesque, M., and J. W. Katcheson. 1963. The influence of variety, soil temperature, and phosphorus fertilizer on yield and uptake by alfalfa. Can. J. Plant Sci. 43: 355-360.

126. Levitt, J. 1956. The Hardiness of Plants. Academic Press Inc., New York. 278 pp.

127. Lindahl, Ivan, R. E. Davis, and W. O. Shepherd. 1949. The application of the total available carbohydrate method to the study of carbohydrate reserves in switch cane (*Arundinaria tecta*). Plant Physiol. 24: 285-294.

128. Lofgreen, G. P., and W. N. Garrett. 1968. A system for expressing net energy requirements and feed values for growing and finishing beef cattle. J. Anim. Sci. 27: 793-806.

129. Lowe, C. C., and J. T. Reid. 1967. Total season feeding values for two growth types of alfalfa grown under two managements. Agron. Abstr. p. 44.

130. Luckett, C. R., and T. J. Klopfenstein. 1970. Leaf-to-stem ratio and composition of alfalfa from five harvesting systems. J. Anim. Sci. 31: 126-129.

131. Markus,D. K., and W. R. Battle. 1965. Soil and plant responses to long-term fertilization of alfalfa (*Medicago sativa* L.). Agron. J. 57: 613-616.

132. Marten, G. C. 1970. Temperature as a determinant of quality of alfalfa harvested by bloom stage or age criteria. Proc. 11th Intern. Grassld. Congr. pp. 506-509.

133. Martin, W. E., and J. E. Matocha. 1973. Plant analysis as an aid in the fertilization of forage crops. B. I. Nutrient criteria for alfalfa. pp. 400-407. In _Soil Testing and Plant Analysis_. L. M. Walsh, and J. D. Beaton (Eds.). Soil Sci. Soc. Amer., Madison, Wisconsin.

134. Mascola, J. J., K. M. Barth, and H. A. Fribourg. 1971. Relation of stage of maturity and plant height to _in vitro_ digestibility of Buffalo alfalfa. Tenn. Farm and Home Sci. # 80. pp. 8-11. Univ. of Tennessee, Knoxville.

135 Matches, A. G., W. F. Wedin, G. C. Marten, Dale Smith, and B. R. Baumgardt. 1970. Forage quality of Vernal and DuPuis alfalfa harvested by calendar date and plant maturity schedules in Missouri, Iowa, Wisconsin, and Minnesota. Wisc. Agric. Expt. Sta. Res. Rpt. 73. 20 pp.

136. Mathur, R. B., and R. L. Pienkowski. 1967. Influence of adult meadow spittlebug feeding on forage quality. J. Econ. Entomol. 60: 207-208.

137. May, L. H. 1960. The utilization of carbohydrate reserves in pasture plants after defoliation. Herb. Abstr. 30: 239-245.

138. Meyer, B. S., and D. B. Anderson. 1952. _Plant Physiology_. B. Van Nostrand Co., New York. 784 pp.

139. Meyer, J. H., and L. G. Jones. 1962. Controlling alfalfa quality. Calif. Agric. Expt. Sta. Bull. 784. 72 pp.

140. Minson, D. J. 1967. The voluntary intake and digestibility in sheep of chopped and pelleted _Digitaria decumbens_ (pangolagrass) following a late application of fertilizer nitrogen. Brit. J. Nutr. 21: 587-597.

141. Minson, D. J., and Milford, R. 1967. The voluntary intake and digestibility of diets containing different proportions of legume and mature pangola grass (_Digitaria decumbens_). Austr. J. Exptl. Agric. Anim. Husb. 7: 546-551.

142. Moe, P. W., J. T. Reid, and H. F. Tyrrell. 1965. Effect of level of intake on digestibility of dietary energy by high-producing cows. J. Dairy Sci. 48: 1053-1061.

143. Moore, J. E., and J. C. Waller. 1971. Procedure for determining voluntary intake and nutrient digestibility of hay with sheep. (Revised). Nutr. Lab., Dept. Anim. Sci., Univ. Fla. Gainesville. 5 pp.

144. Moore, J. E., G. O. Mott, D. G. Dunham, and R. W. Omer. 1972. Large capacity in vitro organic matter digestion procedure. J. Anim. Sci. 35: 232. Abstr.

145. Moore, L. A., H. M. Irvin, and J. C. Shaw. 1953. Relationship between TDN and energy values of feeds. J. Dairy Sci. 36: 93-97.

146. Mott, G. O. 1959. Symposium on forage evaluation. IV. Animal variation and measurements of forage quality. Agron. J. 51: 223-226.

147. Mott, G. O., and J. E. Moore. 1970. Forage evaluation techniques in perspective. Proc. National Conference on Forage Quality, Evaluation and Utilization. Lincoln, Nebr. September 3-4, 1969.

148. Mowat, D. N. 1968. Application and implications of in vitro digestibility technique to plant breeding. Amer. Soc. Agron. Special Publication 13 pp. 85-93.

149. Mowat, D. N., R. S. Fulkerson, W. E. Tossell, and J. E. Winch. 1965. The in vitro dry matter digestibility of several species and varieties and their plant parts with advancing stages of maturity. Proc. 10th Intern. Grassld. Congr., Sao Paulo, Brazil. pp. 801-806.

150. Murata, Y., J. Iyama, and T. Honma. 1966. Proc. Crop Sci. Soc. Japan. 34: 385-390. Cited by R. H. Brown, R. B. Pearce, D. D. Wolf, and R. E. Blaser. Energy accumulation and utilization. pp. 143-166. In Alfalfa Science and Technology. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisc.

151. Nelson, W. L. 1968. Plant factors affecting potassium availability and uptake. pp. 355-383. In The Role of Potassium in Agriculture. V. J. Kilmer, S. E. Younts, and N. C. Brady (Eds.). ASA, CSSA, SSSA, Madison, Wisc.

152. Nelson, W. L., and S. A. Barber. 1964. Nutrient deficiencies in legumes for grain and forage. pp. 143-179. In Hunger Signs in Crops. H. B. Sprague (Ed.). David McKay Co., New York.

153. Nelson, W. W., and J. M. MacGregor. 1957. The effect of time and rate of fertilizer application on the yield, composition, and longevity of alfalfa. Soil Sci. Soc. Amer. Proc. 21: 42-46.

154. Nelson, C. J., and Dale Smith. 1968. Growth of birdsfoot trefoil and alfalfa. III. Changes in carbohydrate reserves and growth analysis under field conditions. Crop Sci. 8: 25-28.

155. Nelson, C. J., and Dale Smith. 1969. Growth of birdsfoot trefoil and alfalfa. IV. Carbohydrate reserve levels and growth analysis under two temperature regimes. Crop Sci. 9: 589-591.

156. Nielsen, H. M., and C. P. Lysgaard. 1956. Relationship between root and top growth and organic root reserves in lucerne. pp. 77-107. The Royal Vet. Agr. Coll. Yearbook, Copenhagen, Denmark.

157. Nutrient Requirements of Dairy Cattle. 4th revised edition. 1971. N.R.C., N.A.S. Table 4, p. 30.

158. Ogden, R. L., and W. R. Kehr. 1968. Proc. 10th Techn. Alfalfa Conf., U.S.D.A., A.R.S. 74-46: 23-37. Cited by Dale Smith (196) loc. cit.

159. Oh, H. K., B. R. Baumgardt, and J. M. Scholl. 1966. Evaluation of forages in the laboratory. V. Comparison of chemical analyses, solubility test and in vitro fermentation. J. Dairy Sci. 49: 850.

160. Oliver, S., and S. A. Barber. 1966. An evaluation of the mechanisms governing the supply of Ca, Mg, K, and Na to soybean roots (Glycine max). Soil Sci. Soc. Amer. Proc. 30: 82-86.

161. Osbourn, D. F. 1967. The intake of conserved forages. pp. 20-28. In Fodder Conservation. R. J. Wilkins (Ed.). Occas. Symp. Brit. Grassld. Soc. No. 3.

162. Osbourn, D. F., D. J. Thomson, and R. A. Terry. 1966. The relationship between voluntary intake and digestibility of forage crops, using sheep. Proc. 10th Intern. Grassld. Congr., Helsinki. pp. 363-366.

163. Pearson, R. W., and F. Adams. 1967. Soil Acidity and Liming. Agronomy 12. Amer. Soc. Agron., Madison, Wisc. 274 pp.

164. Peterson, M. L., and R. H. Hagan. 1953. Production and quality of irrigated pasture mixtures as influenced by clipping frequency. Agron. J. 45: 283-287.

165. Pohlman, G. G. 1946. Effect of liming different soil layers on yields of alfalfa and on root development and nodulation. Soil Sci. 62: 255-266.

166. Pozo, I. M. del. 1963. The effect of cutting treatments on the dry matter production of _Lorium perenne_ L. and _Dactylis glomerata_ L. Versl. Landbouwk. Onderz. No. 69.17, p.74.

167. Prine, G. M. 1966. Alfalfa persistence in Florida. Soil and Crop Sci. Soc. of Fla. Proc. 26: 217-226.

168. Raymond, W. F. 1969. The nutritive value of forage crops. Advanc. Agron. 21: 1-108.

169. Raymond, W. F., and R. A. Terry. 1966. Studies of herbage digestibility by an _in vitro_ method. Outlook on Agric. 5: 60-68.

170. Reid, J. T. 1961. Problems of feed evaluation related to feeding of dairy cows. J. Dairy Sci. 44: 2122-2133.

171. Reid, J. T., W. K. Kennedy, K. L. Turk, S. T. Slack, G. W. Trimberger, and R. P. Murphy. 1959. Effect of growth stage, chemical composition and physical properties upon the nutritive value of forages. J. Dairy Sci. 42: 567-571.

172. Reid, L. R., and G. A. Jung. 1965. Factors influencing the intake and palatability of forages for sheep. Proc. 9th Intern. Grassld. Congr., Sao Paulo, Brazil. pp. 863-869.

173. Rhykerd, C. L., and C. J. Overdahl. 1972. Nutrition and fertilizer use. pp. 437-468. In _Alfalfa Science and Technology_. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

174. Rhykerd, C. L., C. H. Noller, J. E. Dillon, J. B. Ragland, B. W. Crowl, G. C. Naderman, and D. L. Hill. 1967. Managing alfalfa-grass mixtures for yield and protein. Ind. Agric. Expt. Sta. Res. Bull. 839. 7 pp.

175. Richardson, A. E. V., H. C. Trumble, and R. E. Shapter. 1931. Bull. 49 Counc. Sci. Industr. Res. Aust. Cited by M. H. French. 1957. Nutritional value of tropical grasses and fodders. Herb. Abstr. 27: 1-9.

176. Robison, G. D., and M. A. Massengale. 1968. Effect of harvest management and temperature on forage yield, root carbohydrates, plant density and leaf area relationship in alfalfa (_Medicago sativa_ L., cultivar "Moapa"). Crop Sci. 8: 147-151.

177. Robison, G. D., and M. A. Massengale. 1969. Effect of night temperature on growth and development of alfalfa (Medicago sativa L.). J. Ariz. Acad. Sci. 5: 227-231.

178. Robinson, R. R., R. E. Blaser, and H. B. Peterson. 1957. Soil management for pastures. The 1957 Yearbook of Agriculture. pp. 628-633.

179. Ruelke, O. C., and G. M. Prine. 1967. A new look at alfalfa for Florida. Soil and Crop Sci. Soc. of Fla. Proc. 27: 106-114.

180. Ruelke, O. C., and G. M. Prine. 1972. Fertilization and cutting management effects on the yield, quality and persistence of Florida 66 alfalfa. Soil and Crop Sci. Soc. of Fla. Proc. 27: 106-114.

181. Sandal, P. C., and C. L. Garey. 1955. Effect of topdressing permanent pastures with superphosphate on beef yields and distribution of available P_2O_5 in the soil. Agron. Jr. 47: 229-231.

182. Schmehl, W. R., and S. D. Romsdal. 1963. Materials and method of application of phosphate for alfalfa in Colorado. Colorado Agric. Expt. Sta. Bull. 74. 25 pp.

183. Seatz, L. F., and C. O. Stanberry. 1963. Advances in phosphate fertilization. pp. 155-187. In Fertilizer Technology and Usage. M. H. McVickar, G.L. Bridger and L. B. Nelson (Eds.). Soil Sci. Soc. Amer., Madison, Wisconsin.

184. Shirk, G. A., E. M. Kesler, J. W. Bratzler, and J. B. Washko. 1968. Effect of initial cutting date on the yield and nutritive value of mixed hays and their aftermaths. J. Dairy Sci. 51: 1639-1643.

185. Shumard, R. F., D. W. Bolin, and D. F. Eveleth. 1957. Physiological and nutritional changes in lambs infected with the nematodes, Haemonchus contortus, Trichostrongylus colobriformis, and Nematodirus spathiger. Amer. J. Vet. Res. 18: 330-337.

186. Singh, R. N., D. C. Martens, and S. S. Obenshain. 1966. Plant availability and form of residual phosphorus in Davidson clay loam. Soil Sci. Soc. Amer. Proc. 30: 617-620

187. Smith, Dale. 1962. Alfalfa cutting practices. I. Influence of cutting schedule, soil fertility and insect control on yield and persistence of Vernal and Narragansett altalfa. Wisc. Agric. Expt. Sta. Res. Rpt. 11. 11 pp.

188. Smith, Dale. 1964. Chemical composition of herbage with advance in maturity of alfalfa, medium red clover, ladino clover and birdsfoot trefoil. Wisc. Agric. Expt. Sta. Res. Rept. 16. 10 pp.

189. Smith, Dale. 1964. Winter injury and the survival of forage plants. Herb. Abstr. 34: 203-209.

190. Smith, Dale. 1968. (Revised). The establishment and management of alfalfa. Wis. Agric. Expt. Sta. Bull. 542. 22 pp.

191. Smith, Dale. 1969. Influence of temperature on the yield and chemical composition of Vernal alfalfa at first flower. Agron. J. 61: 470-472.

192. Smith, Dale. 1969. Removing and analyzing total nonstructural carbohydrates from plant tissue. Wisc. Agric. Expt. Sta. Res. Rpt. 41, 11 pp.

193. Smith, Dale. 1970. Yield and chemical composition of leaves and stems of alfalfa at intervals up the shoots. J. Agric. Food Chem. 18: 652-656.

194. Smith, Dale. 1970. Influence of temperature on the yield and chemical composition of five forage legume species. Agron. J. 62: 520-523.

195. Smith, Dale. 1971. Levels and sources of potassium for alfalfa as influenced by temperature. Agron. J. 63: 497-500.

196. Smith, Dale. 1972. Cutting schedules and maintaining pure stands. pp. 481-496. In Alfalfa Science and Technology. C. H. Hanson (Ed.). Agronomy 15. Amer. Soc. Agron., Madison, Wisconsin.

197. Smith, Dale. 1973. Influence of drying and storage conditions on nonstructurual carbohydrate analysis of herbage tissue. A review. J. Brit. Grassld. Soc. 28: 129-134.

198. Smith, Dale, and C. J. Nelson. 1967. Growth of birdsfoot trefoil and alfalfa. I. Response to height and frequency of cutting. Crop Sci. 7: 130-133.

199. Smith, Dale and J.P. Silva. 1969. Use of carbohydrate and nitrogen root reserves in the regrowth of alfalfa from greenhouse experiments under light and dark conditions. Crop Sci. 9: 464-467.

200. Smith, Dale, and B. E. Struckmeyer. 1974. Gross morphology and starch accumulation in leaves of alfalfa plants grown at high and low temperatures. Crop Sci. 14: 433-436.

201. Smith, Dale, G. M. Paulsen and C. A. Raguse. 1964. Extraction of total available carbohydrates from grass and legume tissue. Plant Physiol. 39: 960-962.

202. Smith, Dale, M. L. Jones, R. F. Johannes, and B. R. Baumgardt. 1966. The performance of Vernal and DuPuits alfalfa harvested at first flower or three times by date. Wisc. Agric. Expt. Sta. Res. Rpt. 23. 8 pp.

203. Smith, Dale, G. C. Marten, A. G. Matches, and W. F. Wedin. 1968. Dry matter yields of Vernal and DuPuits alfalfa harvested by calendar date and plant maturity schedules in Missouri, Iowa, Wisconsin, and Minnesota. Wisc. Agric. Expt. Sta. Res. Rpt. 37. 10 pp.

204. Smith, G. E., and W. A. Albrecht. 1942. Feed efficiency in terms of biological assays of soil treatments. Soil Sci. Soc. Amer. Proc. 7: 322-330.

205. Smith, L. H., and G. C. Marten. 1970. Foliar regrowth of alfalfa utilizing ^{14}C-labeled carbohydrates stored in roots. Crop Sci.10: 146-150.

206. Smith, L. W., H. K. Goering, D. R. Waldo, and C. H. Gordon. 1971. In vitro digestion rate of forage cell wall components. J. Dairy Sci. 54: 71-76.

207. Saphr, S. L., E. M. Kesler, J. W. Bratzler, and J. B. Washko. 1961. Effects of stage of maturity at first cutting on quality of forages. J. Dairy Sci. 44: 503-510.

208. Spedding, C. R. W. 1954. Effect of a subclinical worm-burden on the digestive efficiency of sheep. J. Comp. Path. 64: 5-14.

209. Steinmetz, F. H. 1926. Winter hardiness in alfalfa varieties. Minn. Agric. Expt. Sta. Techn. Bull. 38. 33 pp.

210. Sullivan, J. T. 1962. Evaluation of forage crops by chemical analysis. A critique. Agron. J. 54: 511-515.

211. Sullivan, J. T. 1966. Studies of the hemicellulose of forage plants. J. Anim. Sci. 25: 83-86.

212. Tan, T. H., and K.Baeumer. 1972. On the relationship between accumulated reserve carbohydrates and regrowth of red clover after cutting. Zacker Pflanzenbau 134 (4): 265-284. (Biological Abstracts # 5253, Nov. 15, 1972.)

213. Technicon AutoAnalyzer II for Kjeldahl N determination. Technicon Industrial Systems. A division of Technicon Instruments Corporation. Tarrytown, New York 10591.

214. Teel, M. R. 1968. Effect of potassium on the organic acid and nonprotein nitrogen content of plant tissue. pp. 165-188. In The Role of Potassium in Agriculture. V. J. Kilmer, S. E. Younts, and N. C. Brady (Eds.). ASA, CSSA, SSSA, Madison, Wisconsin.

215. Terman, G. L., E. C. Doll, and J. A. Lutz, Jr. 1960. Rate, source, time, and method of applying phosphates for alfalfa and legume-grass hay and pasture. Agron. J. 52: 261-264.

216. Terry, R. A., and J. M. A. Tilley. 1963. Exptl. Grassld. Res. Inst., Hurley. 15: 47. cited by W. F. Raymond (169) loc. cit.

217. Terry, R. A., and J. M. A. Tilley. 1964. The digestibility of the leaves and stems of perennial ryegrass, cocksfoot, timothy, tall fescue, lucerne and sainfoin, as measured by an in vitro procedure. J. Brit. Grassld. Soc. 19: 363-372.

218. Tesar, M.B. 1968. Potassium builds alfalfa quality. Better Crops with Plant Food. 52 (1): 6-7.

219. Tilley, J. M. A., R. A. Terry, R. E. Deriaz, and G. E. Outen. 1964. Exptl. Res. Inst., Hurley. 16: 64-67. Cited by W. F. Raymond (169) loc. cit.

220. Tisdale, S. L., and W. L. Nelson. 1966. Soil Fertility and Fertilizers. 2nd Edn. MacMillan Co., New York. 694 pp.

221. Tomlin, D. C., R.R. Johnson, and B. A. Dehority. 1965. Relationship of lignification to in vitro cellulose digestibility of grasses and legumes. J. Anim. Sci. 24: 161-165.

222. Topps, J. H., W. D. C. Reed, and R. C. Elliot. 1964. The effect of season and supplementary feeding on the rumen contents of African cattle grazing subtropical herbage. J. Agric. Sci. 64: 397-402.

223. Troelsen, J.E., and J. B. Campbell. 1959. Nutritional quality of forage crops adapted to southwestern Saskatchewan as determined by their digestibility and dry matter intake when fed to sheep. Can. J. Plant Sci. 39: 417-430.

224. Troelsen, J. E., and J. B. Campbell. 1969. The effect of maturity and leafiness on the intake and digestibility of alfalfas and grasses fed to sheep. J. Agric. Sci., Camb. 73: 145-154.

225. Twamley, B. E. 1960. Variety, fertilizer, management interactions in alfalfa. Can. J. Plant Sci. 40: 130-138.

226. Ueno, M., and Dale Smith. 1970. Growth and carbohydrate changes in the root wood and bark of different sized alfalfa plants during regrowth after cutting. Crop Sci. 10: 396-399.

227. Van Riper, G. E., and Dale Smith. 1959. Changes in the chemical composition of alfalfa, medium red clover, ladino clover, and bromegrass with advance in maturity. Wisc. Agric. Expt. Sta. Res. Rpt. 4. 25 pp.

228. Van Riper, G. E., and F. G. Owen. 1964. Effect of cutting height on alfalfa and two grasses as related to production, persistence, and available soil moisture. Agron. J. 56: 291-295.

229. Van Soest, P. J. 1964. Symposium on nutrition and forage and pastures: New chemical procedures for evaluating forages. J. Anim. Sci. 23: 838-845.

230. Van Soest, P. J. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: Voluntary intake in relation to chemical composition and digestibility. J. Anim. Sci. 24: 834-843.

231. Van Soest, P. J. 1967. Development of a comprehensive system of feed analyses and its application to forages. J. Anim. Sci. 26: 119-128.

232. Van Soest, P. J., and L. H. P. Jones. 1968. Effect of silica in forage upon digestibility. J. Dairy Sci. 51: 1644-1648.

233. Vough, L. R., and G. C. Marten. 1971. Influence of soil moisture and ambient temperature on yield and quality of alfalfa forage. Agron. J. 63: 40-42.

234. Walstrom, R. J., P. A. Jones, and G. F. Gastler. 1970. Effect of phorate for partial control of alfalfa weevil on nutritional values of alfalfa hay. J. Econ. Entomol. 63: 1374-1375.

235. Wang, J. Y. 1960. A critique of heat unit approach to plant response studies. Ecology 41: 785-790.

236. Ward, C. Y., and R. E. Blaser. 1961. Carbohydrate food reserves and leaf area regrowth of orchard-grass. Crop Sci. 1: 366-370.

237. Washko, J. B., and J. W. Price. 1970. Intensive management of alfalfa for forage production. Proc. 11th Intern. Grassld. Congr., Surfers Paradise, Australia. pp. 628-632.

238. Wedin, W. F., A. W. Burger, and H. L. Ahlgren. 1956. Effect of soil type fertilization, and stage of growth on yield, chemical composition, and biological value of Ladino clover (_Trifolium repens_ L.) and alfalfa (_Medicago sativa_ L.). Agron. J. 48: 147-152.

239. Weinmann, H. 1947. Determination of total available carbohydrates in plants. Plant Physiol. 22: 279-290.

240. Weir, W. C., L. G. Jones, and J. H. Meyer. 1960. Effect of cutting interval and stage of maturity on the digestibility and yield of alfalfa. J. Anim. Sci. 19: 5-19.

241. West, S. H., and G. M. Prine. 1960. Alfalfa persistence studies. Soil and Crop Sci. Soc. Fla. Proc. 20: 93-98.

242. White, J. G. H. 1967. Establishment of lucerne on acid soils. pp. 105-114. In _The Lucerne Crop_. R. H. M. Langer (Ed.). A. H. and A. W. Reed Press, Wellington, New Zealand.

243. Wieringa, G. W. 1958. The effect of wilting on butyric acid fermentation in silage. Neth. J. Agric. Sci. 6: 204-210.

244. Willard, C. J. 1951. The management of alfalfa meadows after seeding. Vol. 3: 93-112. In _Advances in Agronomy_. Academic Press Inc., New York.

245. Willard, C. J., L. E. Thatcher, and J. S. Cutter. 1934. Alfalfa in Ohio. Ohio Agric. Expt. Sta. Bull. 540. 146 pp.

246. Willis, W. G., D. L. Stuteville, and E. L. Sorensen. 1969. Effects of leaf and stem diseases on yield and quality of alfalfa forage. Crop Sci. 9: 637-640.

247. Wilsie, C. P. 1962. Crop Adaptation and Distribution. W. H. Freeman and Co., San Fransisco. 448 pp.

248. Wilsou,R. H., and H. J. Evans. 1968. The effect of potassium and other univalent cations on the conformation of enzymes. pp. 189-202. In The Role of Potassium in Agriculture. V. J. Kilmer, S. E. Younts, and N. C. Brady, (Eds.). ASA, CSSA, SSSA, Madison, Wisconsin.

249. Winch, J. E., R. W. Sheard, and D. N. Mowat. 1970. Determining cutting schedules for maximum yield and quality of bromegrass, timothy, lucerne and lucerne/grass mixtures. J. Brit. Grassld. Soc. 25: 44-52.

250. Wolf, D. D. 1973. Test tells when to cut alfalfa. Crops and Soils Magazine. April/May, 1973. p. 18.

251. Wolf, D. D., L. L. Larson, and Dale Smith. 1962. Grass-alfalfa yields and food storage of associated alfalfa as influenced by height and frequency of cutting. Crop Sci. 2: 363-364.

252. Woodhouse Jr., W. W. 1964. Forages need lime, phosphate, and potash. Better Crops with Plant Food. 48 (2): 12-19.

253. Woodman, H. E., and R. E. Evans. 1930. The utilization by sheep of mineral deficient herbage. J. Agric. Sci. Camb. 20: 587-615.

254. Woodman, H. E., and R. E. Evans. 1935. Nutritive value of lucerne. IV. The leaf/stem ratio. J. Agric. Sci. Camb. 25: 578-597.

255. Woodruff, C. M. 1967. Crop response to lime in the midwestern United States. pp. 207-231. In Soil Acidity and Liming. R. W. Pearson, and F. Adams (Ed.). Agronomy 12. Amer. Soc. Agron., Madison, Wisconsin.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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